

ROMANIAN JOURNAL of DIABETES, NUTRITION AND METABOLIC DISEASES

Original Research

CYTOKINE PROFILE AND C-REACTIVE PROTEIN LEVEL IN PATIENTS WITH ISCHEMIC HEART DISEASE ON THE BACKGROUND OF METABOLIC SYNDROME

*Mariia Shchurko, Nataliia Lapovets, Lyubov Lapovets,
Viorika Akimova, Halyna Bashta, Galyna Lebed, Olha
Martianova, Yuliia Stepas, Yevhenii Pysarenko, Natalija
Bojkiv*

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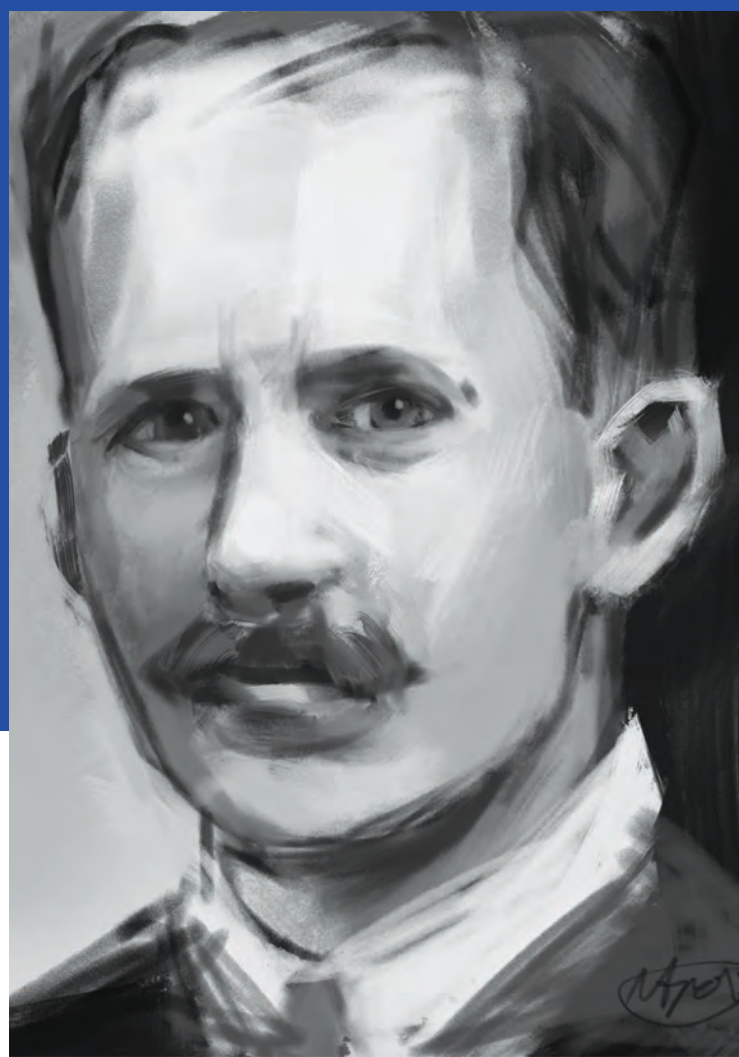
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*Mariana Spătăreanu, MD, PhD student, Amorin Remus Popa, MD
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Romanian Journal of Diabetes, Nutrition and Metabolic Diseases

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Romanian Journal of Diabetes, Nutrition and Metabolic Diseases is the Official Journal
of the **Romanian Society of Diabetes, Nutrition and Metabolic Diseases**

ISSN-L: 2068-8245

print: 2068-8245

online: 2284-6417

Indexed by: IndexCopernicus International • getCITED • SCOPUS • Scirus

Sănătatea Press Group SRL

9C Alexandru Moruzzi street, Bucharest 031152, Romania. Tel.: +40 31 710 88 01, Fax: +40 031 710 88 02

E-mail: office@sanatateapress.ro, www.sanatateapress.ro

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Original Research

Cytokine profile and C-reactive protein level in patients with ischemic heart disease on the background of metabolic syndrome

Mariia Shchurko¹, Nataliia Lapovets², Lyubov Lapovets¹, Viorika Akimova^{1*}, Halyna Bashta¹, Galyna Lebed¹, Olha Martianova¹, Yuliia Stepas¹, Yevhenii Pysarenko², Natalija Bojkiv¹

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Received: 11 November 2020 / Accepted: 24 December 2020

Abstract

Background and aims: In this work, it was aimed to study the levels of interleukins 1 β , 6, 18, and C-reactive protein (CRP) in the blood serum of patients with ischemic heart disease (IHD). **Material and method:** Clinical and laboratory examination of 120 persons from verified IHD was carried. Out of which 60 patients with IHD without metabolic syndrome (Group 1) and 60 patients with metabolic syndrome [(Group 2) (59 male, 1 female)]. Age of the patients are 55 $\pm$ 2 years. The presence of metabolic syndrome was assessed based on the level of triacylglycerol. The obtained laboratory values were compared with the control group, which included 30 healthy individuals. All study subjects were tested for serum levels of IL-1 β , IL-6, IL-18, and CRP. **Conclusions:** It was found that metabolic syndrome in patients with coronary artery disease acts as a trigger for the synthesis of several pro-inflammatory cytokines. Statistically significant increase in IL-1 β (1.5 times), IL-6 (8.3 times), IL-18 (1.4 times), and CRP (1.3 times) in Group 2 compared to Group 1 of patients indicates the presence of an inflammatory process. Determination of the cytokine status of IHD patients can be a biomarker for the development of an inflammatory response.

Keywords: cytokines, C-reactive protein, ischemic heart disease, metabolic syndrome

Background and aims

Cardiovascular disease is a cause of 67% of deaths in Ukraine and globally. Ischemic heart disease (IHD) is the most common type of heart disease, which causes substantial morbidity and incapacitation in the population. Within this context, the problem of IHD is one of the leading healthcare issues of the 21st century [1, 2].

Ischemic heart disease involves damage to the heart muscle caused by impaired blood flow in the coronary arteries. Myocardial ischemia is

synonymous with atherosclerosis. The cause of this condition is the accumulation of atherosclerotic plaques in the vessels due to increased systemic levels of cholesterol (i.e. the development of metabolic syndrome), resulting in narrowing of vascular lumen and inadequate perfusion of various affected organs [1, 3]. The cumulative effect of various components of metabolic syndrome (MS) can be very detrimental since individual risks synergistically augment one another and the overall risk of IHD becomes quite high. Subjects with MS who were 29–79 years of age



and free from any clinical signs of IHD have been often diagnosed with calcification of coronary arteries on their CT scans. Metabolic syndrome includes the disorders accompanied by the development of hypertension (HT), hyperlipidemia, insulin resistance (IR), and abdominal obesity in the patients. Such clinical manifestations are accompanied by the development of immunodeficiency [3, 4]. The increase in plasma levels of non-specific markers of inflammation serves as an indicator of acute-phase responses; moreover, both the amplitude and the nature of this change to some extent depend on the activity of the disease [5]. Potential pro-inflammatory factors include oxidized low-density lipoproteins and pro-inflammatory cytokines [4]. Both pro- and anti-inflammatory cytokines serve as mediators of intercellular interactions; these substances support local inflammation within the atherosclerotic plaque by activating endothelial cells and by inducing the expression of adhesion molecules and the pro-thrombotic activity of the endothelium [4, 6]. The questions regarding the changes in concentrations of novel cardiovascular biomarkers in patients with IHD, which reflect the status of systemic inflammation, remain currently debatable, therefore the studies in this regard are quite promising.

This work aimed to evaluate serum levels of interleukins 1 β , 6, 18, and C-reactive protein (CRP) in patients with IHD.

Material and method

Study design and patients

The authors have performed clinical and laboratory assessments in 120 patients with verified IHD, including 60 IHD patients without a metabolic syndrome (30 males and 30 females) – Group 1 and 60 patients with metabolic syndrome (59 males and 1 female) – Group 2. The mean age of the patients were 55 ± 2 years. The presence of metabolic syndrome was assessed by triacylglycerol levels. The resulting laboratory findings were compared with those in the control group, which enrolled 30 virtually healthy volunteers.

Laboratory, anthropometric, and clinical data collection

Fasting venous blood samples (5 mL each) were obtained from all patients. Blood was sampled into BD Vacutainer plastic blood collection tubes with a dual coagulation activator.

All study subjects were tested for serum levels of IL-1 β , IL-6, IL-18, and CRP. Assessment of interleukin levels was performed using reagent kits manufactured by VECTOR-BEST Ukraine on a STAT FAX 303 plus analyzer. The levels of CRP were assessed using a CRPLX reagent kit by Roche Diagnostics on a COBAS INTEGRA 400 plus automatic analyzer.

Statistical analysis

Statistical analysis of the data obtained was performed using mathematical methods and STATISTICA 8.0 software package (Statsoft, USA) with subsequent analysis of study results. Basic statistics, such as arithmetic mean (M) and standard error of the mean (m) were calculated. Results in the tables have been provided as $M \pm m$. The differences between arithmetic means were considered significant at $p < 0.05$. Each variable was tested for normal distribution using the Shapiro-Wilk test. Depending on experimental conditions and distribution of data, inter-group differences were assessed using a paired or unpaired t-test and non-parametric Mann-Whitney test [7].

Results

As a result of our study, we found statistically significant differences in serum levels of investigational interleukins in study patients with IHD (Table 1).

Serum levels of IL-1 β in patients of Group 1 were within normal. In patients of Group 2, the level of IL-1 β was 1.6 times normal and 1.5 times the level in patients of Group 1.

The level of IL-6 in patients of Group 1 was three times lower than the level in the control group and 8.3 times lower than the level in study patients of Group 2. The serum level of IL-6

Table 1: Serum levels of interleukins 1 β , 6, and 18 in patients with IHD.

Parameters	Groups of subjects		
	Control Group (n = 30)	Group 1 (n = 60)	Group 2 (n = 60)
IL-1 β (pg/mL)	1.55 $\pm$ 0.05	1.67 $\pm$ 0.05 p > 0.05	2.57 $\pm$ 0.05 p < 0.05 p ₁ < 0.05
IL-6 (pg/mL)	2.1 $\pm$ 0.05	0.7 $\pm$ 0.05 p < 0.05	5.82 $\pm$ 0.1 p < 0.05 p ₁ < 0.05
IL-18 (pg/mL)	364 $\pm$ 5.0	333.4 $\pm$ 5.0 p < 0.05	471.7 $\pm$ 5.0 p < 0.05 p ₁ < 0.05

Note: p = the probability of differences compared to findings in the control group; p₁ = the probability of differences compared to findings in patients of Group 1.

Table 2: Serum levels of C-reactive peptide in patients with IHD.

Parameters	Groups of subjects		
	Control Group (n = 30)	Group 1 (n = 60)	Group 2 (n = 60)
CRP (mg/L)	2.8 $\pm$ 0.1	2.99 $\pm$ 0.1 p > 0.05	3.85 $\pm$ 0.1 p < 0.05 p ₁ < 0.05

Note: p = the probability of differences compared to findings in the control group; p₁ = the probability of differences compared to findings in patients of Group 1.

in study patients of Group 2 was 2.8 times higher than the respective level in healthy individuals.

Serum IL-18 level in patients of Group 1 was characterized by the same trends as the levels of previously described cytokines: it was 1.1 times lower than normal and 1.4 times lower than the respective level in Group 2.

As a result of our study, we have found a 1.4-fold increase in CRP levels in study subjects of Group 2 relative to controls (Table 2).

In Group 1, there were no statistically significant differences in CRP levels from the control group; CRP levels in patients of Group 1 were 1.3 times lower than those in patients of Group 2.

Discussion

Therefore, patients with IHD and comorbid metabolic syndrome were found to have substantially elevated levels of investigational interleukins. The presence of metabolic syndrome in such patients leads to the intensification of inflammatory

processes, which was manifest as an increased synthesis of pro-inflammatory interleukins.

Pro-inflammatory cytokines (such as IL-1 β , IL-6, IL-18, etc.) were produced and act upon immunocompetent cells, thereby initiating inflammatory responses. Many members of the research community [8–10] make a point that increased levels of these cytokines reflect the activity and severity of the disease. The effects of cytokines were closely related to physiological (normal) and pathophysiological responses in the body. With that, there is a modulation of both local and systemic defenses.

IL-1 β is a multifunctional cytokine with a wide range of actions. It plays a key role in the development and regulation of general defenses and specific immunity; also, it is one of the first substances to be recruited into the defensive response of the body to various pathogenic factors. Macrophages and monocytes are the principal producers of this cytokine [4].

When influenced by IL-1, endothelial cells of the human vascular system secrete

polypeptides, which are similar to platelet-derived growth factor. These polypeptides may stimulate migration and proliferation of cells and trigger the release of vascular mediators of inflammation; in a setting of significantly increased levels of this cytokine, this may lead to disseminated intravascular coagulation [6, 10].

Both IL-1 and IL-6 initiate the synthesis of acute-phase proteins. Increased levels of IL-1 and IL-6 are associated with repeat coronary events in patients with IHD. Several highly powered studies [11] have shown elevated IL-6 levels to have more important prognostic significance for cardiovascular deaths and other cardiovascular complications compared to C-reactive peptide [5].

Research papers [4, 6, 11, 12] present the evidence of increased IL-2, IL-4, IL-6, IL-12, and IL-18 levels in IHD patients with metabolic disorders as opposed to healthy individuals; along with this, IL-6 levels were even higher in patients with myocardial infarction. IL-6 is one of the multifunctional cytokines, i.e. those that stimulate the proliferation of T-cells, macrophages, and endothelial cells [11]. It also has effects in hematopoietic precursor cells and serves as a growth and differentiation factor for B-cells, hepatocytes, and neurons. IL-6 helps activate endothelial cells, monocytes, and execute pro-coagulant reactions. IL-6 modulates immunological processes, inflammation, proliferation, and apoptosis [4, 11, 13].

Testing for IL-6 levels is playing an important role in the diagnosis of congestive heart failure. Circulating cytokines stimulate the secretion of acute-phase proteins, such as CRP. Under normal conditions, inflammation is very precisely regulated by cytokines, which may have both pro-inflammatory and regulatory properties, thereby inhibiting inflammation. Such regulation is very important in preventing excessive tissue damage. If the causative agent of inflammation is not eliminated, the inflammation may persist and become chronic, which leads to significant tissue damage [5, 13].

Thus, IL-18 is known to be induced by stress signals (of neurogenic or bacterial origin). Some authors [14, 15] argue that stress-induced IL-18 release may enhance the IFN- γ /IL-18 cycle:

after the first wave of IL-18-induced synthesis of IFN- γ by lymphocytes, the de novo synthesized IFN- γ , in turn, stimulates monocytes/macrophages, leading to an increase in their activity, which, in part, promotes the formation of IL-18 [16]. Thereby, IL-18 not only stimulates the synthesis of IFN- γ but also modulates its functional activity. The researchers have demonstrated [16, 17] that expression of NK cells also occurs under the influence of IL-18; either independently or through IFN- γ , this interleukin stimulates the initiation of apoptotic processes.

Thus, as seen in our studies, an increase in profiles of cytokines (IL-1, IL-6, and IL-18) was observed in subjects with IHD in a setting of metabolic syndrome. According to worldwide statistics, patients with comorbid metabolic syndrome and stable angina die of IHD twice as often as patients with normal metabolism. Adipose tissue is known to be a source of energy in the body and to have additional endocrine functions. Adipocytes secrete more than 50 biologically active substances, which in turn affect metabolic activity in multiple organs, either directly or indirectly, through neuroendocrine mechanisms, i.e. via pituitary hormones, insulin, and catecholamines [17]. The problem of early detection of coronary disease is very pressing for patients with metabolic syndrome since it defines the possibility of timely correction of symptoms [18, 19]. Assessment of interleukin levels in this patient population will allow for early diagnosis of developing cardiovascular disease.

Conclusions

In patients with IHD, metabolic syndrome serves as a trigger for the synthesis of some pro-inflammatory cytokines. The statistically significant increases in IL-1 β (1.5-fold), IL-6 (8.3-fold), IL-18 (1.4-fold), and CRP (1.3-fold), which were observed in IHD patients with metabolic syndrome (as opposed to the group of IHD patients without metabolic disorders) suggest the presence of inflammation. Assessments of cytokine status in patients with IHD are potential biomarkers of a developing inflammatory response.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

Peculiarities of carbacetam effect on the processes of fibrinolysis and proteolysis in the brain of rats with neurodegeneration induced by type 2 diabetes mellitus

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Received: 4 October 2020 / Accepted: 16 November 2020

Abstract

Introduction: Diabetic neurodegeneration is formed with underlying type 2 diabetes mellitus (T2DM) which is an independent factor promoting development of cognitive disorders. Considering an important role of GABA receptors in the development of neurodegenerative processes, the issue concerning carbacetam effect produced on proteolytic and fibrinolytic mechanisms of the cerebral cortex and hippocampus with experimental simulation of T2DM has become of a certain interest. **Materials and Methods:** Type 2 diabetes mellitus was simulated by streptozotocin in the dose of 30 mg/kg injected through the peritoneum of rats kept during 30 days on a high-fat diet with a free access to fructose solution. **Results:** Under conditions of pathological process development the activity of the main groups of proteolytic enzymes intensify in the cerebral cortex and hippocampus. Carbacetam administration to rats with diabetes mellitus promotes reduction of proteolysis processes which might be associated with pharmacodynamics of the drug. **Conclusions:** The data obtained experimentally substantiate reasonability of pathogenic correction by means of carbacetam in case of disorders of proteolytic and fibrinolytic systems activity in the cerebral cortex and hippocampus induced by T2DM.

Keywords: carbacetam, fibrinolysis, proteolysis, type 2 diabetes mellitus

Introduction

Diabetes mellitus (DM) is one of the most common endocrine pathologies in the world. The disease is characterized by a chronic course and development of complications in various organs and systems resulting in disability of capable of working individuals [1]. Diabetic neurodegeneration is formed with underlying type 2 diabetes mellitus (T2DM) which is an independent factor promoting development of cognitive disorders.

Brain activity considerably depends on the balance between neural activity and its metabolic requirements. Neurodegenerative processes are associated with these mechanisms respectively [2]. Considering a dynamic character

of the brain activity and considerable metabolic requirements of the bioelectric-active nervous tissue, microcirculation of the brain must be very sensitive to the tissue it supplies. A direct innervation of the microvascular endothelium by means of the receptors of gamma amino butyric acid (GABA) neurons is confirmed anatomically [3]. GABA activity enables to maintain and improve metabolic, neurotrophic and energy supply of the brain by means of control over the nervous-vascular units [4], which dysfunction promotes disturbance of the hematoencephalic barrier integrity. Damages or poorly functioning barrier is more susceptible to inflammatory reactions including microglia and astrocytes which can promote advance of neurodegenerative processes.



Moreover, decreased GABA concentration in the brain will promote increasing pathological role of other neurotransmitters, glutamate with calcium compromised neurons [5], oxidative stress, disturbance of the membrane integrity in particular, resulting in swelling and activation of intracellular protease.

Under hyperglycemia conditions free radicals are known to be formed in the process of glucose “self-oxidation” during formation of the final products of accelerated glycosylation. Further they react with unsaturated compounds disturbing the structure of enzymatic proteins and lipids of the cellular membranes [6]. These processes activate proteolysis and lipid peroxide oxidation resulting in changes of physical-chemical properties of the biological membranes. It leads to destruction of membranous lipids where hydrolytic enzymes play the main role. An excessive formation of free radicals followed by the damage of the neuron membranous structures and DNA results in functional disorders of the nerve cells [7].

Considering the importance of GABA role in the receptors development of neurodegenerative processes, the issue concerning carbacetam effect produced on proteolytic and fibrinolytic mechanisms of the cerebral cortex and hippocampus with experimental simulation of T2DM has become of a certain interest.

Objective

To study carbacetam effect, as a modulator of GABA-receptors, produced on the fibrinolysis and proteolysis in the brain of rats with neurodegeneration induced by T2DM.

Materials and Methods

The experiments were conducted on non-linear laboratory albino male rats with their body weight of 0.18–0.20 kg, kept under standard vivarium conditions with natural alternation of day and night, according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific

Purposes (Strasbourg, 1986); European Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions regarding the protection of animals used for experimental and other scientific purposes, and the Order of the Ministry of Health of Ukraine № 690 of 23 September 2009.

Type 2 diabetes mellitus was simulated by streptozotocin (Stz) in the dose of 30 mg/kg on the citrate buffer (pH = 4.5) injected through the peritoneum of rats kept during 30 days on a high-fat diet with a free access to fructose solution (200 g/L) [8]. On the 11th week after Stz injection the group of rats with DM (7 rats) received a course (14 days) of carbacetam injections through the peritoneum in the dose of 5 mg/kg in the volume 1 mL of 0.9% NaCl solution/0.1 kg of the body weight. The groups of comparison, that is, control group and the group of rats with simulated pathology (7 rats in each) received a solvent through the peritoneum in the similar regimen.

Euthanasia of rats was performed under light ether narcosis. The brain was removed cool and carefully washed with cool 0.9% NaCl solution. The hippocampus and cerebral cortex were removed according to the stereotaxic [9]. Brain homogenates were prepared in 0.05 M tris-HCl buffer (pH 7.4).

Proteolysis/fibrinolysis was investigated according to the method using the set of reagents Simko Ltd. The state of proteolytic activity was determined on the basis of reaction with azoalbumin (low molecular proteinolysis), azocasein (high molecular proteinolysis) and azocollagen (collagenolysis). Fibrinolytic activity was examined evaluating the degree of homogenate staining due to plasmin formation in the presence of ϵ -aminocaproic acid (non-enzymatic fibrinolytic activity [NFA]) or without it (total fibrinolytic activity [TFA]). Enzymatic fibrinolytic activity (EFA) was determined by the difference between TFA and NFA. Proteolysis and fibrinolysis were estimated in E440/hour/mg of tissue, where E440 – extinction index for activity [10].

The results of the study were statistically processed applying parametric Student t-criterion. In case normal distribution was lacking Mann-Whitney U-criterion was applied. The differences were considered reliable with $p < 0.05$. Point

estimate of the results was represented in the form of mean values and standard mean error ($M \pm m$).

Results

The studies conducted demonstrated (Figures 1, 2) that under conditions of neurodegeneration induced by T2DM proteolytic activity of the cerebral cortex was characterized by 22.1% increase of enzymatic azoalbumin breaking up, 6.7% increase of azocaseinolysis and 65.5% increase of azocol. A similar tendency was observed while studying proteolytic activity of the hippocampus (Figures 3, 4) of rats with diabetes mellitus. Thus, the index of azoalbuminolysis did not differ reliably from that of the control, azocaseinolysis 26.8% increased and azocollagen – 67.5%. Fibrinolysis in the cerebral cortex of rats with DM (Figure 5) was characterized by the increase of total fibrinolytic activity (TFA) at the expense of enzymatic fibrinolytic activity (EFA). At the same time, TFA 9.9% increased and EFA 14.2% increased concerning the control values. Fibrinolysis in the hippocampus (Figure 6) differed from that of the control by 26.7% increase of EFA index.

Carbacetam administration to rats with DM promoted reduction of the proteolytic processes in the cerebral cortex according to the indices of azoalbumin enzymatic breaking out (6.1% decrease) and azocollagenolysis (8.5% decrease) in comparison with the values of the simulated pathology. Azocaseinolysis and azocololysis in the hippocampus of rats 7.3% and 9.6% decreased, respectively. And carbacetam administration in this case did not change reliably the indices of fibrinolysis in the cerebral cortex and hippocampus of rats with T2DM.

Discussion

Under conditions of pathological process development the activity of the main groups of proteolytic enzymes and synthesis of protein peroxide oxidation products intensify in the cerebral cortex and hippocampus, which is indicated in our previous publications [8]. Carbacetam administration to rats with DM promotes reduction of

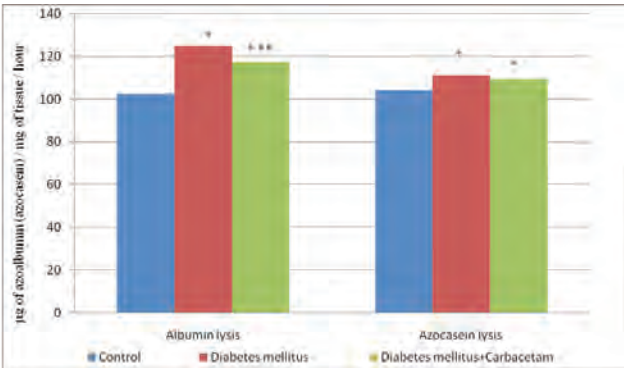


Figure 1: The effect of carbacetam on the rates of proteolysis of the cerebral cortex of rats under conditions of neurodegeneration induced by T2DM ($M \pm m$, $n = 7$).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

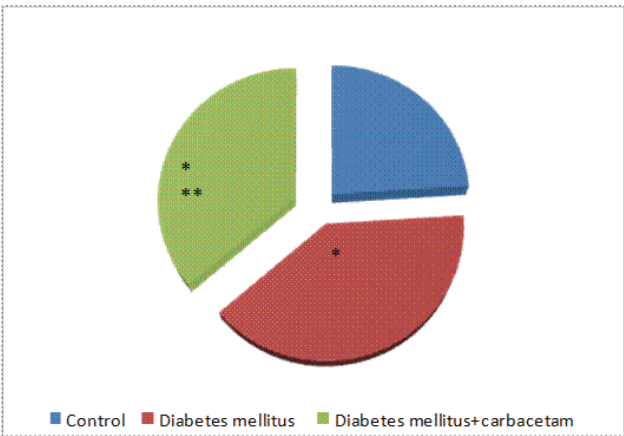


Figure 2: The effect of carbacetam on azocollagen lysis of the cerebral cortex of rats under conditions of neurodegeneration induced by T2DM ($M \pm m$, $n = 7$).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

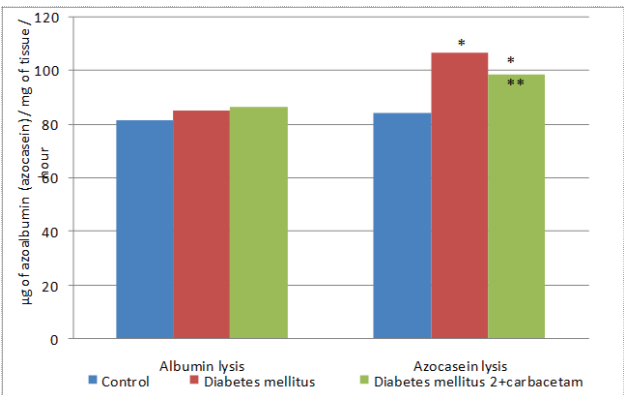


Figure 3: The effect of carbacetam on the proteolysis of the rat hippocampus under conditions of neurodegeneration induced by T2DM ($M \pm m$, $n = 7$).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

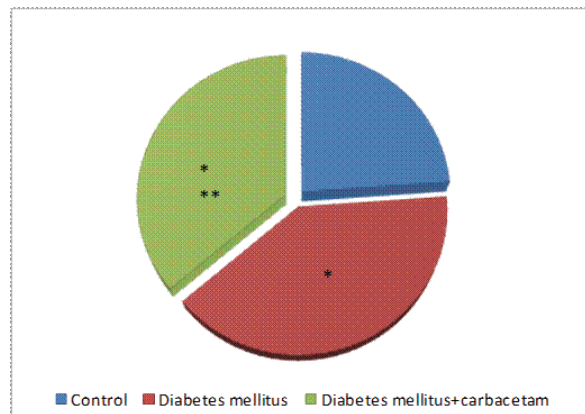


Figure 4: The effect of carbacetam on azocollagen lysis of the rat hippocampus under conditions of neurodegeneration induced by T2DM ($M \pm m$, $n = 7$).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

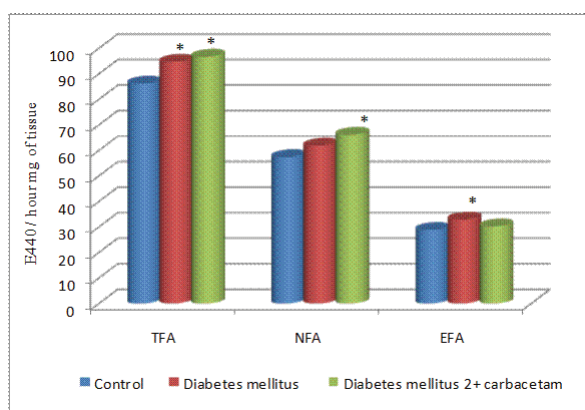


Figure 5: The effect of carbacetam on fibrinolysis of the cerebral cortex of rats under conditions of neurodegeneration induced by T2DM ($M \pm m$, $n = 7$).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

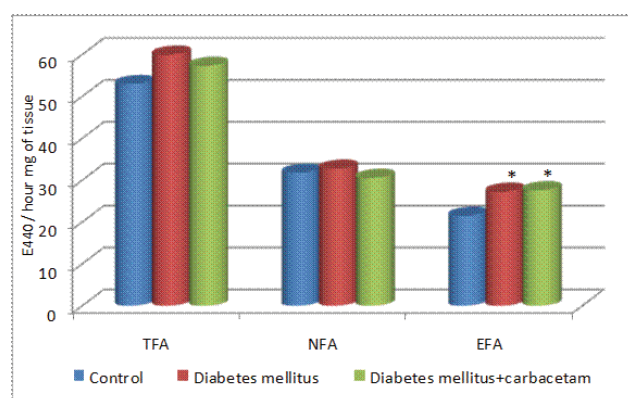


Figure 6: The effect of carbacetam on fibrinolysis of the hippocampus of rats under conditions of neurodegeneration induced by T2DM ($M \pm m$, $n = 7$).

Notes: *Reliability of difference compared with the control group of rats, **Reliability of difference compared with the group of rats with T2DM.

proteolysis processes which might be associated with pharmacodynamics of the drug, since modulation of GABA receptors controls both direct and indirect penetration of calcium ions into the neurons through the astrocytes and glial network. The neurons become hyperpolarized. Their hyperpolarization plays a crucial role in a long inhibition of the synaptic transmission [11, 12]. During hyperpolarization phase, calcium channels, protected by energy, are blocked and therefore protecting neurons against toxicity of calcium ions and giving a chance for them to eliminate their excessive amount [13, 14]. The processes of mitochondria oxidation slow down respectively (lipid and protein peroxide oxidation) [15] resulting in decrease of proteolytic mechanisms [16]. Thus, due to removal of toxic calcium excess from the cells mitochondrial production of adenosine triphosphate increases quickly. An excessive irritability of neurons decreases. The processes leading to neuron apoptosis become slower.

One more probable carbacetam effect is regulation of neurotransmitter level and maintaining of metabolism in neurons [8]. The brain activity considerably depends on the balance between neuron activity and its metabolic requirements. At the same time, a great amount of lactate is released into the extracellular space which is utilized through the astrocyte network and saves neuron activity during glucose deficiency [17]. First of all, due to metabolic disorders the structure and functions of erythrocytes change – they become spherical in shape, their ability to deformity decreases resulting in reduced blood flow and endothelial damage [18]. Erythrocytes adsorb plasma proteins, fibrinogens and fibrin on their surface decreasing superficial negative charge. It results in intensification of adhesion and aggregation of erythrocytes. Metabolic disorders produce a negative effect of the platelets as well. The effect of long hyperglycemia on the endothelial cells results in increased production of fibrinolytic factors, intensification of thrombotic activity (reduced synthesis of prostacyclin and increased synthesis of thromboxane A₂ by platelets) [19]. It promotes greater aggregation of platelets, vascular spasm and damage of the capillary epithelium. All the above processes disturb the integrity of the hematoencephalic barrier, which functioning

considerably depends on the activity of GABA-receptors [4, 20]. Thereafter, modulation with carbacetam will promote retention of the barrier integrity and reduction of inflammatory processes. Due to these mechanisms both fibrinolysis and proteolysis slow down. This is what we have observed in our studies.

Therefore, administration of carbacetam to rats with neurodegeneration induced by T2DM is pathogenically substantiated by the results of the studies conducted.

Conclusions

Development of neurodegeneration induced by T2DM is characterized by considerable changes of fibrinolytic and proteolytic activity of the cerebral cortex and hippocampus tissues leading to imbalance between the tissue indices. Administration of carbacetam to rats with diabetes mellitus during 14 days decreases collagenolysis, enzymatic fibrinolytic activity in the cerebral cortex, low molecular proteinolysis in the hippocampus; the values of proteolysis in the cerebral cortex decrease according to the findings of enzymatic breaking up of azoalbumin and azocollagenolysis, azocasinolysis and azocololysis in the hippocampus of rats. The data obtained experimentally substantiate reasonability of pathogenic correction by means of carbacetam in case of disorders of proteolytic and fibrinolytic systems activity in the cerebral cortex and hippocampus induced by T2DM.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

Complex treatment of patients with complicated syndrome of diabetic foot and sepsis

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Received: 6 January 2021 / Accepted: 18 February 2021

Abstract

Introduction: Efforts to improve survival in sepsis are based on a series of urgent medical interventions. The article presents the data obtained in the process of complex treatment of patients, in which sepsis was caused by complicated diabetic foot syndrome. **Materials and Methods:** The article presents materials on the retrospective study of treatment results of 1678 patients with complicated diabetic foot syndrome. In 146 patients (8.7%) the disease was burdened with sepsis. **Results:** A common feature of the treatment strategy for patients in both groups was surgical intervention, the administration of antibiotic therapy (ABT) and infusion-transfusion therapy. With the help of developed algorithms, it was managed to improve the results of septic patients' treatment. **Conclusions:** The applied tactics of treating patients with sepsis has allowed to reduce the frequency of execution of high amputations and postoperative complications, increase the number of "small" amputations, which led to a reduction in mortality, shortening the length of patients' stay in the hospital.

Keywords: amputation, antibiotic therapy, diabetic foot syndrome, sepsis

Introduction

At the beginning of the new century, purulent-inflammatory processes in patients with diabetes and sepsis remain one of the most pressing and unresolved problems of modern medical science. With the development of sanitary and hygienic measures, development and implementation of new antibacterial drugs, the incidence curve has come gone down. On the contrary, there is a tendency to the increase of sepsis incidence [1, 5, 10].

Surgical complications of diabetes mellitus, namely purulent-necrotic lesions of the

tissues of the foot are among the most common pathologies in the clinics of purulent-septic surgery. Since about 8–12% of patients with sepsis are with impaired carbohydrate metabolism. The features of diagnosis and treatment of sepsis in patients with complicated DFS (diabetic foot syndrome) is a significant medical problem. Unsatisfactory results of treatment of complicated DFS require a comprehensive multidisciplinary approach to solving this problem [8, 9, 19, 20].

It is known that without elimination of the center of a purulent infection in patients with sepsis the most powerful intensive care is doomed. An equally important component



that ensures the success of treatment is a full infusion-transfusion and inotropic support [4, 15, 17]. The latter statement is embodied in the “Sepsis-3” guidelines [2, 3, 6, 7, 11–14, 16, 18].

The aim of the work

The article is aimed to improve the treatment results of patients with purulent-necrotic lesions of the tissues of the foot and sepsis on the background of complicated DFS.

Materials and Methods

Under our examination and treatment from 2010 to 2020 there were 1678 patients with complicated DFS, who were in a purulent-septic center with beds of diabetic foot in the non-profit municipal enterprise “City Hospital № 3” in Zaporizhzhia (Institute of Sepsis of SI “Zaporizhzhia Medical Academy of Postgraduate Education of the Ministry of Healthcare of Ukraine”) (Department of outpatient, purulent-septic surgery and ultrasound). In 146 patients (8.7%) the disease was aggravated by sepsis.

The definition of “complicated DFS” combines purulent-necrotic lesions of the foot-ulcers, abscesses, phlegmon, purulent tendovaginitis, purulent arthritis, gangrene, as well as diabetic osteoarthropathy.

The patients were divided into two clinical groups:

1. The first group (comparison group) included 724 patients with complicated DFS who received treatment for the period from 2010 to 2016 (beginning). In 71 patients the disease was aggravated by sepsis – they formed the 1 subgroup.
2. The second (main) group consisted of 954 patients (with 75 cases of sepsis included into the 2 subgroup) who were treated during 2016–2020.

Patients in both groups had type 2 diabetes, the average duration of which was (12.4 ± 2.3) years.

Criteria for inclusion in the study: type 2 diabetes, the presence of complicated DFS with purulent-necrotic processes of the tissues of the foot.

Exclusion criteria: type 1 diabetes, DFS with intact skin (stage 0 according to the Wagner classification), patients on hemodialysis.

Certain difficulties arose in the gradation of patients with sepsis, as according to the “Sepsis-3” guidelines the concept of “severe sepsis” is excluded. All cases of sepsis were carefully analyzed according to the new bedside clinical indicator of gSOFA, which is characterized by a respiratory rate of more than 22 per 1 min., impaired consciousness (Glasgow coma scale) and an average blood pressure of 100 mmHg or less. With the help of above said indicators, patients of subgroups 1 and 2 were randomized.

Determination of the qualitative composition of the flora and the sensitivity of the selected cultures to antibiotics was performed automatically (Vitek 2 Compact – France). The technical capabilities of the method allowed to perform the identification of aerobic and facultative anaerobic microorganisms, in addition to non-spore-forming anaerobic microorganisms, as well as to detect the presence of the MRSA gene.

Thus, 57 (80.3%) patients with sepsis and 14 (19.7%) with septic shock (SS) were assigned to subgroup 1, and 53 (69.3%) patients with sepsis and 22 (30.7%) with septic shock – subgroup 2. Statistical data processing was performed taking into account the principles of evidence-based medicine, calculations were performed using a software package for statistical data analysis “STATISTICA 6.1”.

Research Results

Common features of the treatment strategy for the patients of both groups were surgery, antibiotic therapy (ABT) and infusion-transfusion therapy.

Patients underwent various surgical interventions: necrectomy, necro- and sequestrectomy, dissection of abscesses and phlegmon,

amputation of fingers, feet and limbs. Extensive opening of the purulent center, excision within viable tissues and drainage during deep purulent-necrotic processes were performed. The amputation of the foot according to Shopar was preferred in common purulent-inflammatory lesions of the foot with the involvement of several toes. Further treatment of wounds was performed using modern dressings depending on the stage of the wound process. Criteria for assessing the effectiveness of treatment from the postoperative wound were: the speed of wound healing, maintaining the lower limb support ability, the number of repeated surgical interventions, the presence of certain complications. The average length of patients' stays in the hospital, general and postoperative mortality were also taken into account.

In the comparison group, wound cleansing and the appearance of the first granulations were recorded at (6.3 ± 0.23) days, in the main group, wound cleansing occurred at a faster rate – at (5.7 ± 0.21) days ($p < 0.05$).

In most cases, patients with sepsis had wet gangrene of the lower extremity. Therefore, they were performed high amputations at the level of the thigh on vital signs in an urgent manner. In patients with sepsis in middle age, amputations were performed at the level of the upper third of the tibia (according to Burgess). Successful implementation of this operation reduces postoperative mortality and creates opportunities for prosthetics and improvement of life quality. Burgess amputation at the level of the tibia (4 patients of subgroup 1 and 7 patients of subgroup 2) preserves the knee joint and increases the chances of successful prosthetics compared to amputation at the hip level. It is not advisable to perform amputations in this group of patients at a lower level of tibia.

The anterior and posterior skin and muscle flaps were excised. The length of the front was 2 cm, the back – up to 15 cm. The stump of the tibia was made 12–13 cm long, and the fibula was 5 cm longer than the level of the tibia. One of the main stages of the operation was considered the excision of the soleus muscle. Muscle removal was performed due to impaired blood flow and frequent complications in the form of necrosis of

the latter. The superficial fascia of the calf muscles was sutured to the tibial bed and the medial periosteum of the tibia. The posterior flap was moved anteriorly to the rounded end of the tibial stump and sutured with a short anterior flap. The postoperative wound was drained by active aspiration.

High amputation was performed at the level of the middle or upper third of the thigh according to Pirogov or fascio-plastic method, with cutting of two skin-fascial flaps and active drainage of the postoperative wound.

These operations were effective because the septic process did not progress.

Verification of the etiological structure of the foci of infectious-inflammatory process in patients with purulent-inflammatory processes of the foot of diabetics and sepsis is a significant problem at the present stage, which is confirmed by numerous domestic and foreign scientific publications. Therefore, the microbiological characteristics of the bacterial flora of purulent-necrotic foci in patients with complicated DFS and sepsis are the basis for the formation of a program of rational ABT and assessment of the wound process.

ABT in patients with complicated DFS and the development of sepsis should provide the maximum therapeutic effect with minimal impact on the body of a patient suffering from severe comorbidities.

Technological features of antibacterial therapy:

- empirical ABT should be performed before obtaining the result of microbiological research, its effectiveness depends on the correct choice of drug and its effect on all potential pathogens;
- directed ABT can be assigned only after receiving the results of seeding. If necessary to re-correct ABT taking into account the selected microflora and its sensitivity to antibacterial drugs;
- targeted ABT was performed in a short course (10–12 days) to obtain a clear clinical result;
- in severe infection with sepsis, the duration of ABT is 2–4 weeks, and in the presence of osteomyelitis it can be increased to 6 weeks.

Infusion therapy (IT) in patients with DFS was started immediately in patients with hypotension or organ hypoperfusion. It was one of the primary measures to support hemodynamics and, above all, cardiac output.

In patients with sepsis and septic shock (SS), the approach to infusion therapy has slightly changed. Infusion therapy was regarded as a first-line intervention to restore systemic hemodynamics and increase oxygen delivery to meet the needs of septic patients.

Septic patients with insufficient tissue perfusion, hypotension, or signs of hypovolemia received an initial fluid volume of 30 mL/kg body weight of crystalloid solutions during the first 3 hours of resuscitation, followed by infusion under systematic monitoring of the patient's hemodynamic status. One of the targets was mean arterial pressure (MAP) ≥ 65 mmHg. Another target was the normalization of arterial lactate concentration.

Despite the conduct of IT in patients of both groups, inotropic support is important in the stabilization of hemodynamic parameters, which provides, along with limiting hemodynamic disorders, an adequate level of tissue perfusion. Drugs that provide inotropic support include epinephrine, norepinephrine, dopamine and dobutamine.

After the start of IT, the volume of subsequent infusion was determined depending on the patient's hemodynamic status.

After 72 hours (deescalation or "deresuscitation" phase), which usually occurs in 96 hours or after restoration of hemodynamic stability, attempts were made to achieve a negative fluid balance or to limit intravenous intensive care, or to increase fluid excretion through diuresis activation.

Thus, the monitoring of intensive care was as follows: blood pressure and heart rate (at normal values) – no indications for massive infusions; daily fluid balance (close to zero during admission to the intensive care unit); central venous pressure is no longer considered an informative indicator; determination of saturation of mixed venous blood with oxygen (ScvO₂) and lactate (at normal values) – no indications for massive infusions.

The most reliable test is to determine the reactivity of the organism to the introduction of fluid (organisms that respond and do not respond). A bolus of 500 mL of isotonic crystalloid is administered: if cardiac output and stroke volume increase by 13–15%, the infusion should be continued, if not vasopressors and cardiotonics are indicated.

Discussion

There are no unambiguous criteria for discontinuation of ABT (decision-making is based on the dynamics of local and systemic inflammatory response, wound condition, eradication of pathogens, the degree of contamination of the wound, normalization of leukocyte formula, C-reactive protein, decreased procalcitonin).

Here are the schemes of antibiotics: levofloxacin – intravenously, 0.5 g 2 times a day + metronidazole, intravenously, 0.5 g three times a day; piperacillin / tazobactam – intravenously, 4.5 g 3–4 times a day; cefaperazone / sulbactam – intravenously, 4.0 g 2–3 times a day; ceftaroline – intravenously, 0.6 g 2 times a day; ertapenem – intravenously, 1.0 g 1 time per day; imipenem / cilastatin – intravenously, 0.5–1.0 g three times a day; meropenem – intravenously, 1–2.0 g three times a day. Diagnosis of septic shock requires the appointment of imipenem / cilastatin 1.0 g 4 times a day intravenously or meropenem 2.0 g 3–4 times a day intravenously + linezolid 0.6 g 2 times a day intravenously or vancomycin 15 mg / kg 2 times a day.

The administration of intensive infusion-transfusion therapy and inotropic support in patients with sepsis under the "Sepsis-3" guidelines helped to stabilize the condition of patients and prepare them for surgery. Out of 30 patients from the secondary comparison group, 8 (27.7%) patients who did not undergo surgery died. In the main group – 3 (18.8%) patients respectively. Thus, mortality in patients with SS, who were not operated, decreased by 8.9% ($\chi^2 = 5.63$; $p < 0.0177$).

Introduction of optimized methods of purulent-necrotic processes treatment in patients with complicated DFS allowed to reduce

the number of repeated surgical interventions from 26.4 to 20.9% ($p < 0.05$), increase the number of organ-saving operations ($\chi^2 = 5.93$; $p < 0.0149$), to maintain the capacity of the lower extremity in 92.4% of cases in the main group against 82.3% in the comparison group ($\chi^2 = 5.70$; $p < 0.0169$), to reduce the average duration of treatment in the hospital by 4.7 ± 0.24 days ($t = 4.36$; $p < 0.05$).

Conclusions

1. The introduction of the proposed complex therapy, allowed to influence the final indicators of treatment: to reduce mortality in patients with sepsis from 36.1% (13 patients) in the comparison group to 8.3% (3 patients) in the main group ($p < 0.05$), and in septic shock from 72.7% (16 patients) to 56.3% (9 patients) accordingly.
2. Analysis of the obtained data shows that the application of the developed treatment algorithms allowed to significantly ($p < 0.05$) reduce the frequency of high amputations and postoperative complications, increase the number of "small" amputations, maintain the lower limb support ability, which led to reduced mortality and reduced duration of patients' stay in a hospital.

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Ethic Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

Correlation of Glycated Hemoglobin and Glucose levels after Oral Glucose Tolerance test in gestational diabetes patients: A Cross Sectional study

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Received: 31 January 2021 / Accepted: 15 March 2021

Abstract

Gestational diabetes mellitus (GDM) is a prevalent and potentially serious condition which may put both mothers and neonates at risk. The current recommendation for diagnosis is the oral glucose tolerance test (OGTT). OGTT is a cumbersome test that is time consuming. So we want to test the utility of HbA_{1c} as a screening tool in pregnancy for GDM. **Aim:** This study aimed to determine the usefulness of HbA_{1c} test as a diagnostic tool for GDM as compared to the traditional criteria based on the OGTT. **Materials and Methods:** This study was done among 156 pregnant women of the age group of 20–40 years with gestational period of 24–28 weeks attending the Obstetrics and Gynecology OPD of Chettinad Hospital and Research Institute, Chengalpattu from July 2019 to December 2019. **Results:** The HbA_{1c} values will be correlated with OGTT values using Pearson's correlation analysis and there was significant correlation between HbA_{1c} and post glucose values (p value<0.05). ROC curve analysis done for HbA_{1c} showed an area under the curve of 0.569. **Conclusions:** HbA_{1c} may have a role in the diagnosis of GDM, but further studies among large populations might be required to approve the use of HbA_{1c} instead of OGTT in the diagnosis of GDM.

Keywords: Glycated Hemoglobin, HbA_{1c}, Glucose Tolerance Test, Gestational Diabetes Mellitus

Background and Aim

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset of first recognition during pregnancy [1]. Gestational diabetes mellitus is a prevalent and potentially serious condition that may lead to adverse effects in both mothers and neonates [2]. The prevalence of this condition has increased tremendously over the past few years [3, 4]. Studies are showing that Indian women are having 11 times more risk of developing diabetes when compared to the women in other countries [5]. The prevalence of GDM in Tamil Nadu is 22% which is higher as compared to other parts of the country [6].

There are many criteria for diagnosing GDM. American Diabetes Association had

recommended a two-step approach to diagnose GDM where in first step a 50 g glucose is given as GCT and followed by 75 g or 100 g glucose test [7]. WHO recommended 75 g glucose test, with diagnostic criteria as >126 mg/dL or 200 mg/dL as diagnostic [8].

After the HAPO (Hyperglycemia and Pregnancy Outcome) study, IAPDG gave a recommendation for diagnosing GDM where single dose of 75 g of glucose is given and GDM is diagnosed if one among the following values are above the normal range. Fasting >92 mg/dL, 1 hour ≥180.0 mg/dL, or 2 hour ≥153 mg/dL [9].

In a developing country like India, where there are approximately 27 million pregnancies every year, the number of women approaching the health care facilities at early pregnancy is as low as 50–70%.



Early detection of this condition may lead to reduction of risk factors and complications during pregnancy. GDM causes macrosomia, pre-eclampsia, still birth need for cesarean section and among neonates it causes hypoglycemia and respiratory distress.

HbA_{1c} is a marker which provides an insight into the blood glucose control levels for the past 6–8 weeks, therefore HbA_{1c} can reflect the mean blood glucose levels of early pregnancy [10]. Previous studies shed light on the relationship between HbA_{1c} and adverse pregnancy outcomes [11].

Presently oral glucose tolerance test (OGTT) is accepted as a diagnostic criteria for GDM by international organizations, but it has lot of drawbacks like it requires 8 hours fasting, extensive patient preparation is required, lacks reproducibility, its time consuming, and unpalatable. When compared to OGTT, HbA_{1c} can be measured at any time of the day, has less biological variations and has high reproducibility. However the predictions of some severe events by early maternal HbA_{1c} levels is yet unclear [12].

Aim

This study aimed to determine the usefulness of HbA_{1c} test as a diagnostic tool for GDM as compared to the traditional OGTT method.

Materials and Methods

Ethical committee approval

This study was conducted after obtaining consent from Institute Ethical Committee. It was a cross-sectional study done among 156 pregnant women of the age group of 20–40 years attending the Obstetrics and Gynecology OPD of Chettinad Hospital and Research Institute, Chengalpattu from July 2019 to December 2019 for a period of 6 months. **Inclusion criteria:** All the patients who came to perform OGTT between the gestational period of 24–28 weeks were included in the study. **Exclusion criteria:** Those patients on steroid, anemic patients, patients having associated renal disease or hemoglobin variants and those

who were not willing for the study were excluded from the study.

Laboratory, anthropometric and clinical data collection

Previous obstetric history and family history was taken from all patients. BMI was calculated using the formula weight in kilogram/height in meter². About a 5 mL of blood was drawn from all the patients in violet colored vacutainer tubes for assessing the levels of HbA_{1c} levels and complete blood count. Another 5 mL blood was drawn in red topped vacutainer to assess the levels of thyroid hormones.

We followed DIPSI (Diabetes in pregnancy study group of India) guidelines for diagnosing GDM in our hospital. All the pregnant women were given 75 g of anhydrous glucose mixed in 300 mL of water to drink over 5–10 minutes irrespective of their previous meal status. After 2 hours they were advised to come again and give their blood sample for taking post prandial blood sugar (PPBS) levels. If the PPBS values were ≥ 140 mg/dL, they will be worked up for GDM. HbA_{1c} was measured using chromatographic method using D-10 machine in Biochemistry laboratory. Thyroid hormones like free T₃, free T₄ and TSH (thyroid stimulating hormones) were measured using CLIA (Chemiluminescence immune assay) method in UNICEL DXI machine in Biochemistry laboratory. Complete blood count was measured in pathology laboratory in Beckman Coulter LH 780 machine, total count, platelet count, RBC count and PCV (packed cell volume) were measured using Impedance method. Neutrophils, lymphocytes, eosinophils, basophils, lymphocytes, and monocytes were measured using colorimetric method. MCV, MCHC, MCH and RDW were calculated using formulas. MCV values were calculated using the formula $PCV/RBC \times 10$, MCH using $Hb/RBC \times 10$, MCHC using $Hb/PCV \times 100$. Hemoglobin was measured using cyanmethemoglobin method.

Statistical analysis

The mean $\pm$ SD of all the variables were taken. The sample was divided based on the post

glucose values into two groups as those with values above and >140 mg/dL. Independent sample t test was done between the two groups. Correlation analysis was done between HbA_{1c} and OGTT values.

Results

Mean±SD of all the variables were taken (Table 1). The 156 samples were divided into two groups based on the non-fasting OGTT values as those above and below the levels of 140 mg/dL. Independent sample t test was done to find out between the two groups. There was significant difference in the height, non-fasting glucose levels and HbA_{1c} between the two groups (p value < 0.05) (Table 2).

Correlation analysis between HbA_{1c} and non-fasting OGTT values showed significant correlation values (r value -0.28) (p value = 0.0003) (Figure 1).

Table 1: Shows the mean±SD of all the variables.

Variables	Mean	SD
Height (m)	157.08	4.38
Weight (kg)	62.1	10.3
BMI (kg/m ²)	25.18	4.2
Hemoglobin (g/dL)	11.156	1.3596
Post glucose	120.017	23.0700
HbA _{1c}	5.075	0.4115

ROC curve was done for HbA_{1c} and the area under the curve was found out to be 0.569 (Figure 2).

Discussion

Gestational diabetes mellitus (GDM) is a condition associated with many obstetric and other complications like macrosomia and pre-eclampsia. Women having GDM during pregnancy have a greater chance of developing type 2 diabetes mellitus in the future. So accurate diagnosis is required to correctly identify subjects with GDM. This study will be a unique one where the criteria under DIPSI guidelines for diagnosing GDM is being compared with HbA_{1c}.

DIPSI guideline used for diagnosing GDM among pregnant women has been approved by the Ministry of Health AND Family Welfare [13]. It follows a procedure which is more economical and more feasible for the women in India where in rural areas the hospitals will be far away from the villages and pregnant women will have to travel long distances and do the OGTT test in fasting will be difficult. So a non-fasting tolerance test would be more acceptable for a country like India.

HbA_{1c} is a marker used for the diagnosis of diabetes mellitus. Its use in the diagnosis of GDM is still under debate.

Studies done by Shobha et al. has a shown a relationship between HbA_{1c} and unfavorable pregnancy outcomes [14]. Another study done by

Table 2: Independent sample t test to compare the variables between pregnant women with post glucose levels less than and more than 140 mg/dL.

Variables	Pregnant women with post glucose values <140 mg/dL		Pregnant women with post glucose values >140 mg/dL		p value
	Mean	SD	Mean	SD	
Age (years)	25.1	3.66	25.7	4.14	0.345
Height (cm)	156.9	4.42	155.45	3.4	0.03
Weight (kg)	61.57	10.48	61.95	10.1	0.84
BMI	24.7	4	25.27	4.04	0.39
Hemoglobin (%)	11.12	1.37	11.34	1.32	0.0001
Non-fasting glucose (mg/dL)	112.3	16.19	156.9	13.2	0.0001
HbA _{1c} (%)	5.05	0.39	5.12	0.5	0.334

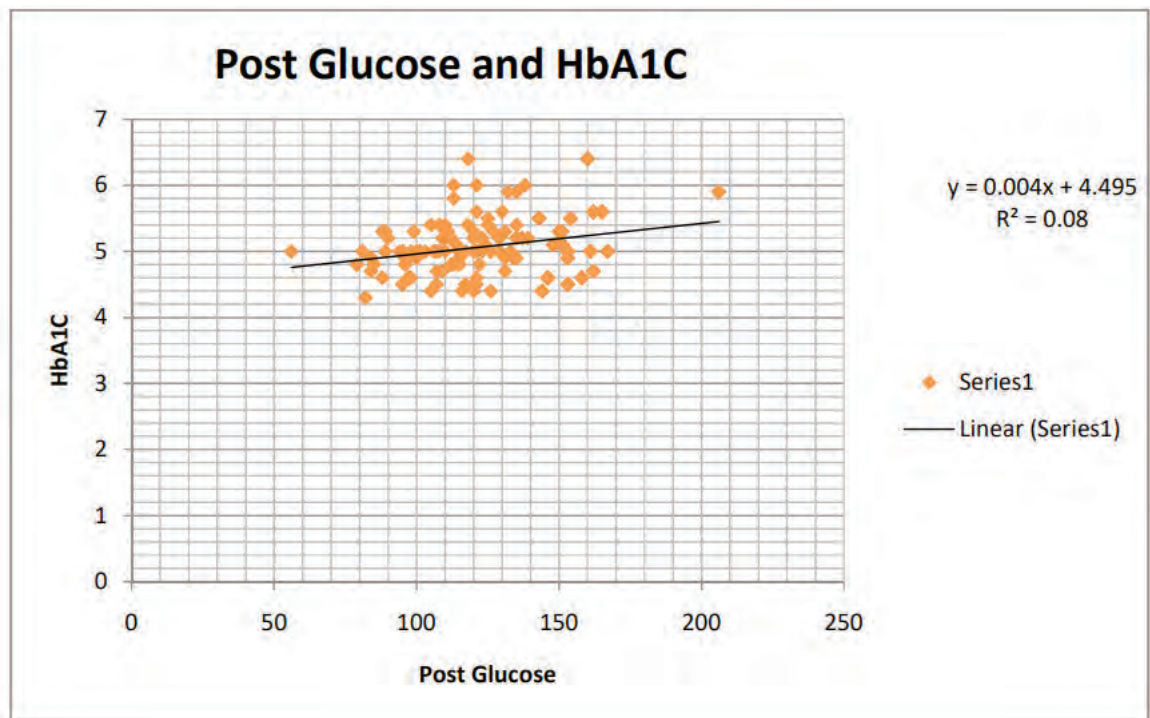


Figure 1: Pearson's correlation analysis between post glucose and HbA_{1c}.

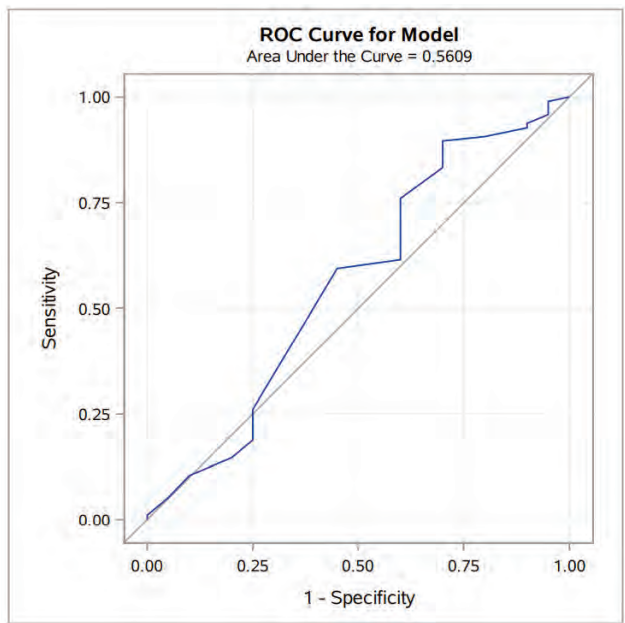


Figure 2: Showing the ROC curve for HbA_{1c}.

Bhavadharaini *et al.* showed that HbA_{1c} levels are associated with the incidence of macrosomia [15].

There was no significant difference in the values of HbA_{1c} between women whose post glucose values were more or less than 140 mg/dL. In our study HbA_{1c} has shown a significant positive correlation between non-fasting glucose values (*r* value -0.28) (*p* value = 0.0003).

HbA_{1c} levels changes throughout pregnancy due to the changes in the hemoglobin levels due to dilution. In this study the ROC curve analysis for HbA_{1c} has shown an area under the curve of 0.569 which is on par with the study done by Agarwal *et al.* comparing HbA_{1c} with screening GDM by WHO criteria showed an area under the curve of 0.54 (95% CI 0.48–0.61) [16].

Rajput *et al.* and Sevket *et al.* compared HbA_{1c} levels with screening GDM by IADPG criteria showed an area under the curve of 0.683, when they screened 607 Indian women and 0.697 (95% CI 0.645–0.745) among 339 Turkish women done respectively [17, 18].

In the present study we did not find any differences in the levels of HbA_{1c} between GDM subjects and normal women, even though there was a significant correlation between HbA_{1c} levels and non-fasting glucose taken as a whole.

So the role of HbA_{1c} in the diagnosis of GDM remains unclear and invites further research in this area.

Conclusion

HbA_{1c} has shown a significant role in the diagnosis of GDM, but still requires studies on a

large population to establish its use instead of OGTT in the diagnosis of GDM.

Acknowledgement

We would like to thank the Chettinad Academy of Research and Education for giving us the support for doing this project.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

Association between glycated hemoglobin and vitamin D levels in type 2 diabetes mellitus

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Received: 9 March 2021 / Accepted: 21 April 2021

Abstract

Background and Aims: Type 2 diabetes mellitus (T2DM) is one of the most common chronic disorders, affecting millions of people around the world. Vitamin D may enhance secretion of insulin from the beta cells of pancreas, increase glucose uptake by the peripheries and decrease systemic inflammation. Hence the study aims to find out the role of vitamin D deficiency in pathogenesis of diabetes mellitus. **Materials and Methods:** A total of 52 T2DM patients were included in the study. The mean level of vitamin D and glycated hemoglobin (HbA_{1c}) were assessed. Fundoscopic evidence was collected to look for evidence of diabetic retinopathy. For that routine urine check was done to look for evidence of proteinuria, which indicating diabetic nephropathy. Statistical analysis was done using SPSS software. **Conclusion:** Our study indicated that there is an inverse relationship between HbA_{1c} and serum vitamin D levels in diabetics. There is increase in the microvascular complications of T2DM with decrease in vitamin D levels.

Keywords: glycated hemoglobin, microvascular complications, type 2 diabetes mellitus, vitamin D

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common chronic disorders, affecting millions of people around the world [1]. It is more common in the developed and developing world owing to obesity, sedentary lifestyle and multiple co-morbidities. Various epidemiological studies suggest individuals with T2DM being asymptomatic, unaware of the symptoms for up to a decade or present with one or more complications of diabetes to health care facilities before a diagnosis of diabetes is made. The most common complications are associated with micro and macro vascular blood vessels, which impair the quality of life significantly, and also increase mortality. Glycated hemoglobin (HbA_{1c}) portrays

the high glucose levels led to vascular complications. Challenges were faced to track day-to-day variation in the glucose level and henceforth the control of diabetes. Therefore, HbA_{1c} is a more reliable indicator of the glycemic status over the last 60–90 days. Hemoglobin (Hb) consists of two globin chains attached to a heme molecule. Majority of adult Hb is comprised predominantly of HbA subtype, with small amounts of HbA2 and HbF. HbA is composed of components: A₀, A_{1a}, A_{1b}, and A_{1c}. A_{1c} is the component with majority of glycation [2, 3]. The RBC membrane is very permeable and intracellular glucose rises in hyperglycemia. Non-enzymatic glycation of the N-terminal valine leads to glycation of HbA_{1c}, which is most commonly affected by age, environment, genetic, hematologic and



illness-related factors. This genetic variation is known as “glycation gap”.

Vitamin D (calciferol) deficiency has taken a resurgence all around the world in the recent years among the other four fat soluble vitamins (A, D, E, K) [4–6]. Vitamin D is an important micronutrient with hormone like action, which is vital for bone mineralization and calcium and phosphorous metabolism. However, there is a lot of recent evidence in various studies portraying its effects in several disorders like cancer, cardiovascular and respiratory illnesses, autoimmune diseases and infections [4, 5]. Vitamin D may enhance secretion of insulin (an anabolic hormone) from the beta cells of pancreas. Increased glucose uptake by the liver in a chronic setting leads to dysfunction of the beta cells of pancreas and the vicious cycle continues, which eventually decrease the insulin receptor auto-phosphorylation and increase the reactive oxygen species (ROS), all these augmenting the insulin resistance (IR). Vitamin D has been found to play a role in T2DM through altering the mechanisms such as IR, beta cell dysfunction and chronic inflammation (these are the hallmarks of T2DM) [7–12]. Henceforth, vitamin D deficiency has found to have a vital role in pathogenesis of diabetes mellitus [13]. Majority of patients with T2DM may have lower serum vitamin D levels [7–9]. Many studies have shown an inverse relationship between vitamin D levels and glycemic control [10, 14–16]. Also, the presence of microvascular complications has been found to be inversely related to vitamin D levels [17–21]. The purposes of this study were to assess the association between glycated hemoglobin and vitamin D levels in T2DM, and also to determine whether there is any association of the microvascular complications with change in HbA_{1c} and vitamin D levels.

Material and Methods

Study design and patients

This is a clinical, prospective and observational study of patients with T2DM. This study was approved by the Institutional Ethical

Committee of NITTE (Deemed to be University). Based on inclusion criteria – patients diagnosed as T2DM, symptoms of diabetes plus RBS $\geq$ 200 mg/dL or FBS $\geq$ 126 mg/dL or HbA_{1c} $\geq$ 6.5% or 2 hour plasma glucose $\geq$ 200 mg/dL during an oral glucose tolerance test, consulting patients; exclusion criteria – patients likely to have vitamin D deficiencies like rickets, osteomalacia, malabsorption, chronic renal failure, cirrhosis of liver, non-consulting patients. The study was conducted in patients with T2DM at the Justice K. S. Hegde charitable hospital, NITTE (Deemed to be university), Deralakatte, Mangalore. A total of 52 study subjects were included in the study.

Laboratory, anthropometric and clinical data collection

All necessary blood tests including HbA_{1c} levels and serum vitamin D level were measured. Fundoscopic evidence was collected to look for evidence of diabetic retinopathy. For that routine urine check was done to look for evidence of proteinuria, which indicating diabetic nephropathy. The sample size and the technique of estimation of proportion was calculated using the below formula.

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d^2}$$

Where, α – Level of significance

d – Precision

P – Anticipated Proportion

Statistical analysis

Statistical analysis was performed using SPSS 15.0 and Epiinfo 6 software packages. Frequency tables, pie charts or bar charts were used to display percentage for categorized variables and continuous variables will be shown as mean $\pm$ SD and error bar charts. To test the significance of association between categorized variables, Chi square test, Mann–Whitney test and ANOVA test were used. $p \leq 0.05$ was considered as significant.

Results

A total of 52 patients were included in the study. Of these majority of the patients (31%) were in the age group of 30–40 years, 27% of the patients belonging to 51–60 year age group, 17% belongs to the age group of 61–70 years, 15% were in the age group of 41–50 years followed by 10% in the age group above 70 years (Figure 1). Among them 54% were female and 46% were male. The study found only 6% patients were seen to have normal vitamin D level, where as 94% of the subjects were seen to have low level of vitamin D. Majority of the study subjects had good (40%) and moderate (35%) glycemic control whereas, 25% of them had poor prognosis of glycemic control. The present study also investigates the presence and absence of microvascular complications such as diabetic retinopathy, nephropathy and neuropathy in the 52 study subjects. Among them, 38% of the study subjects (20 patients) had diabetic retinopathy, of which 13 had NPDR (non-proliferative diabetic retinopathy) and 7 had PDR

(proliferative diabetic retinopathy). Similarly, 35% of the study subjects (18 patients) had diabetic nephropathy as evidenced by the presence of proteinuria, and 11 patients (21%) were found to have symptoms of diabetic neuropathy. Table 1 detailed the comparison of parameters studied between the study subjects.

The mean level of vitamin D and its association with HbA_{1c} was found significant (Table 2). It has seen that higher the HbA_{1c}, lower the vitamin D level (Figure 2). The significant negative correlation was calculated between vitamin D and HbA_{1c} level, it is found to be –735 (Pearson correlation) (Figure 3). Alongside, the level of HbA_{1c} had an inverse relationship with the presence of microvascular complication was statistically significant, which is depicted in Figure 4. There was an inverse relationship between the level of vitamin D and presence of microvascular complication (not statistically significant). However, on regression analysis, it was found that inverse association of HbA_{1c} with vitamin D levels was statistically significant.

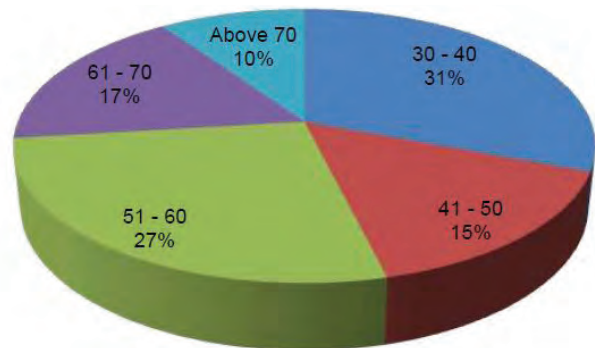


Figure 1: Age wise distribution of patients in percentage.

Table 1: Baseline characteristics of type 2 DM patients.

Parameters	In ratio
Male: Female (in number)	24:28
Vitamin D levels (Normal: Low) (in ng/mL)	3:49
HbA _{1c} level (<7:7–9:>9) (in g/dL)	21:18:13
Diabetic retinopathy (present:absent)	20:32
Diabetic nephropathy (present:absent)	18:34
Diabetic neuropathy (present:absent)	11:41

Table 2: Mean vitamin D level and its association with glyco Hb.

Glyco Hb %	N	Mean Vit D (ng/mL)	Std. dev	95% Confidence Interval for Mean		ANOVA test (p value)	
				LowerBound	UpperBound		
<7	21	25.84	9.04	21.73	29.96	0.000	HS
7–9	18	14.76	4.96	12.29	17.22		
>9	13	8.51	5.07	5.45	11.57		
Total	52	17.67	9.91	14.91	20.43		

Where p value <0.001 is considered as significant. HS indicates highly significant.

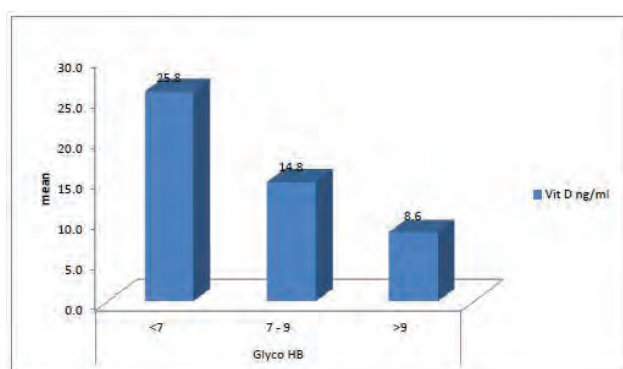


Figure 2: Mean vitamin D level and its association with Glyco Hb.

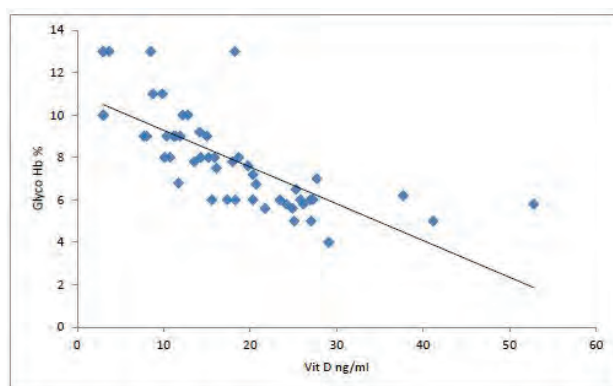


Figure 3: Scatter diagram depicting the association between Glyco Hb and Vitamin D levels.

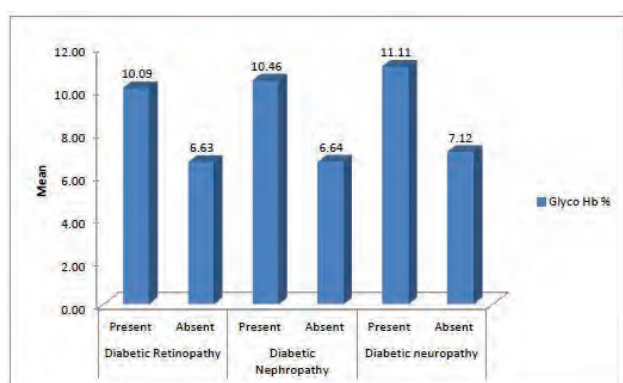


Figure 4: Glyco Hb levels and its association with Micro vascular complications.

Discussion

In our study, 52 patients of T2DM were interviewed and investigated to look for the association between HbA_{1c} and vitamin D levels. We also evaluated their implications on the microvascular complications of T2DM, namely diabetic

retinopathy, diabetic nephropathy and diabetic neuropathy. The gender distribution was almost equal in our study and majority of the patients were belonged to the age group of 30–40 followed by 51–60 years. This was similar to a study by Niyati et al. where, correlation between vitamin D and HbA_{1c} in T2DM patients was estimated for over 100 patients, of which 54 were males and 46 were females [22].

In our study, it was found that only 6% of the patients had normal vitamin D level, which majorly effects on HbA_{1c} levels and their correlation between diabetic and non-diabetic adults. A study conducted by Ahlam et al. [16] involved 299 patients of diabetes in different age groups and only 23 (7.7%) were found to have sufficient vitamin D levels. Khalida et al. [23] conducted a study on 165 patients titled association of vitamin D deficiency with poor glycaemic control in diabetic patients of whom 34 (20%) of the patients had adequate vitamin D levels. The main drawback of our study is the lack of control population and a small sample size. However, the very low percentage of population with normal vitamin D levels has to be looked into further [24]. In our study, majority of the patients had good (40.4%) and moderate (34.6%) glycaemic control whereas 25% of them had poor glycaemic control. A study conducted by Giacomo et al. [15] on 715 patients had 83% of patients with good glycaemic control. Our study also shows significant inverse association between HbA_{1c} and vitamin D levels, where HbA_{1c} is inversely related to serum vitamin D levels in T2DM. Similarly, the study by Ahlam et al. [16] showed the inverse relationship on 184 study subjects to see the effect of vitamin D and HbA_{1c} levels, and their correlation between diabetic and non-diabetic adults. Hypovitaminosis D in patients with T2DM – a relation to disease control and complications, a clinical study on 136 patients by Hala et al. showed that serum vitamin D levels correlated negatively with HbA_{1c} [26]. While in our study there was an inverse relationship between the mean vitamin D level and the occurrence of microvascular complications of T2DM, i.e. diabetic retinopathy, diabetic nephropathy and diabetic neuropathy [27–29]. However, the study conducted by Nikhil et al. [19] on 50 patients of which 25 had microvascular

complications, also showed a similar inverse but statistically insignificant relationship between the two. In a study conducted by Celil *et al.* [25] on 557 patients with T2DM and 112 controls, also found inverse but statistically insignificant association between vitamin D levels and the presence of microvascular complications. However, the report by few studies showed a statistically significant relationship between the microvascular complications of T2DM and serum vitamin D levels [17, 18, 30].

Conclusion

Vitamin D deficiency is highly prevalent among patients with T2DM. Low vitamin D levels were found to be associated with increased microvascular complications of T2DM. So, it is warranted to measure vitamin D levels among diabetics as it is a correctable risk factor. Furthermore, there is also a significant inverse relationship between the levels of HbA_{1c} and vitamin D levels, depicting the importance of glycemic control. Therefore, additional large-scale studies are required to establish if vitamin D supplementation will improve glycemic control among diabetics.

Conflict of interest

The authors declare no conflict of interest.

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Original Research

Association of atherogenic indices with metabolically unhealthy obesity phenotype

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Received: 22 April 2021 / Accepted: 4 June 2021

Abstract

Background and Aims: Obesity is a risk factor for diabetes and cardiovascular disease. Clinical studies have identified two subtypes of obesity: metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUHO), the latter being associated with an increased risk of mortality from any cause or cardiovascular (CV) events. Therefore, for better phenotyping, several atherogenic indices such as atherogenic index and lipid accumulation product have been used that can be calculated at the routine control in order to detect people at high cardiovascular risk. **Materials and Methods:** This was a retrospective study. A total of 163 obese participants (n = 118 metabolically unhealthy subjects with a mean $\pm$ SD body mass index (BMI) of 37.8 ± 5.7 kg/m²) took part in this study. Body composition was assessed by electrical bioimpedance; fasting blood glucose, lipid profile, creatinine, transaminases and serum insulin were obtained using standard protocols. **Results:** The frequency of metabolic syndrome was 72.8%. Patients with MUHO were significantly older, with a higher weight, BMI, waist circumference and waist-hip ratio compared to MHO. All atherogenic indices were statistically significantly higher in patients with MUHO. **Conclusions:** We described several clinical parameters that are accessible in medical offices, which can help the clinician in better phenotyping and identifying MUHO, in order to implement preventive measures to reduce cardiovascular risk.

Keywords: electrical bioimpedance, insulin resistance, metabolic syndrome, phenotyping

Background and Aims

Obesity has reached epidemic proportions and according to the World Health Organization (WHO), worldwide, over 650 million adults were obese in 2016 [1]. It is well-known that obesity is an independent risk factor for type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) [2, 3]. Clinical studies have identified two obesity phenotypes: “metabolically healthy obesity” (MHO), which includes obese persons with a lower risk of metabolic complications, and

“metabolically unhealthy obesity” (MUHO), a group with an increased risk for all-cause mortality or cardiovascular (CV) events [4]. The concept of metabolic health has been recommended since 2000, especially to identify patients with obesity and insulin sensitivity [5]. To date, there is no clear definition of MHO. Therefore, according to the literature, depending on the parameters used, MHO prevalence ranges from 6% to 75% [6]. The most often used definition is the absence of any metabolic disorder and CV disease in patients with a body mass index (BMI) ≥ 30 kg/m² [7].



Also, some metabolic health definitions include insulin resistance indices, inflammatory markers, or cardiorespiratory fitness [8]. Regarding the pathophysiology of metabolic syndrome, it can be classified into 5 subtypes: lipid dominant, vascular dominant, adiposity dominant, insulin resistance dominant, and other risk factors [9].

For a better assessment of obesity phenotypes, and also to predict the risk of CVD, clinical studies have tried to introduce different indices that can be calculated with routinely assessed parameters. These parameters can be considered a plus in the daily practice of the clinician. Lipid accumulation product (LAP) is one of the validated markers of visceral obesity, and the atherogenic index (AI) can be used as a marker in the diagnosis of subclinical atherosclerosis [10, 11]. High levels of non-HDL cholesterol are associated with an increased risk of heart disease. Non-HDL cholesterol to HDL cholesterol (non-HDL-C/HDL-C) ratio is a suitable predictor for metabolic syndrome, insulin resistance, coronary heart disease, and non-alcoholic steatohepatitis (NASH) [12]. Also, total cholesterol/HDL-cholesterol ratio is correlated with ischemic heart disease risk [13]. LDL-C/HDL-C ratio can predict the progression of intima-media thickness (IMT) better than HDL-C or LDL-C alone [14]. Hypertensive waist (HW) is a concept of combination of clinical risk factors that could predict the presence of metabolic syndrome [15]. Although many studies have shown the association of the above mentioned indices with mortality and CVD, few studies have investigated them according to the obesity phenotype.

In this context, the objective of this research was to compare the atherogenic indices in MUHO and MHO and to assess their association with the MUHO phenotype.

Materials and Methods

This was a retrospective study in which were collected data from patients presenting for nutritional assessment in a private clinic. The participants included in this analysis presented for nutritional assessment and counseling for obesity (BMI ≥ 30 kg/m²), age ≥ 18 years, and

available laboratory investigations for plasma glucose, lipid profile, and insulinemia. The exclusion criteria included a previous diagnosis of diabetes mellitus and pregnancy. The final sample for the current analysis consisted of 163 obese participants fulfilling the inclusion criteria, without any exclusion criteria. The study followed the guidelines set by the Declaration of Helsinki.

Data collected and measurements

From patients' medical charts, data on age, gender, anthropometric parameters (weight, height, waist circumference, hip circumference), results of body composition analysis, results of laboratory investigations for plasma glucose, lipid profile, and insulinemia were collected. The BMI was calculated as weight (kg)/ square of height (m), and the waist-hip ratio (WHR) as waist circumference/hip circumference [16]. The body composition analysis was performed by bioelectrical impedance analysis using an InBody 720 device (Biospace Co., South Korea). The parameters evaluated by bioelectrical impedance analysis were visceral fat area, percentage of body fat, skeletal muscle mass, and body fat mass (BFM). Plasma glucose, lipid profile (total cholesterol and HDL cholesterol, serum triglycerides [TG]), and insulinemia were assessed in fasting conditions in the institution's laboratory using a fully automated analyzer (Cobas Integra 400 Plus, Roche). In those with TG levels below 400 mg/dL, LDL cholesterol was calculated using the Friedewald formula: total cholesterol-HDL cholesterol-TG/5. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) formula = (fasting insulin [μ UI/mL] $\times$ fasting plasma glucose [mg/dL])/405. Metabolic syndrome (MS) was defined according to the International Diabetes Federation (IDF) criteria [17]. HTW was defined as the concomitant presence of systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg or a personal history of treated hypertension and the presence of an abdominal circumference ≥ 80 cm in women and ≥ 94 cm in men [15]. The following atherogenic indices were calculated: non-HDL cholesterol,

lipid accumulation product (LAP), atherogenic index (AI), total cholesterol/HDL cholesterol ratio, LDL cholesterol/HDL cholesterol ratio, and non-HDL cholesterol/HDL cholesterol ratio. Non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol. LAP was calculated using the sex-specific formulas proposed by Kyrou *et al.*: [Waist circumference (cm) – 65] × [TG (mmol/L)] for men and [Waist circumference (cm) – 58] × [TG (mmol/L)] for women [18]. AI was assessed as $\log [\text{TG}/\text{HDL cholesterol}]$ [19].

The participants were classified into 2 cardiometabolic phenotypes based on the presence of metabolic syndrome: MHO (BMI ≥ 30 kg/m² and no metabolic syndrome) and MUHO (BMI ≥ 30 kg/m² plus metabolic syndrome).

Statistical analysis

For statistical analysis, SPSS-PC 20.0 (SPSS Inc., Chicago, IL, USA) was used. Data were presented as means and standard deviation for continuous variables with normal distribution, as median (quartile 1; quartile 3) for continuous variables with non-normal distribution, and as number (percentage) for categorical variables. To compare the variables between the MHO and MUHO groups, we used the Student t-test and the median test for continuous variables, and the chi-square test for frequencies. Unadjusted and adjusted logistic regression in the whole sample was employed to assess the variables associated with the presence of MUHO in our sample. A *p* value below 0.05 was statistically significant.

Results

This study evaluated 83 women and 80 men, with a mean age of 38.1 ± 11.2 years, a mean BMI of 37.2 ± 5.5 kg/m², and a mean visceral fat area (VFA) of 168.0 ± 46.6 cm². The frequency of metabolic syndrome was 72.8% and that of HW, 46.6%. Of the whole sample analyzed, 118 patients (72.4%) had MUHO. This phenotype was more frequent among men than women (*p* = 0.005). Patients with MUHO were significantly older and had a significantly higher weight, waist

circumference, and WHR than those with MHO (*p* < 0.001 for all). For the lipid profile, as compared to patients with MHO, those with MUHO had lower HDL cholesterol levels (50.2 mg/dL vs. 40.8 mg/dL, *p* < 0.001) and higher triglycerides (102.0 mg/dL vs. 170.0 mg/dL, *p* < 0.001); no difference between groups was observed for total and LDL cholesterol. Also, patients with MUHO had significantly higher fasting plasma glucose (*p* < 0.001), fasting insulinemia (*p* = 0.016), HOMA-IR (*p* = 0.016), VFA (*p* = 0.002) (Table 1).

All atherogenic indices assessed were statistically significantly higher in patients with MUHO compared to those with MHO. In patients with MUHO vs. those with MHO, LAP was 106.5 vs. 55.4; atherogenic index was 3.9 vs. 2.4 (Table 2).

In the unadjusted logistic regression analysis, all indices were statistically significantly associated with the presence of MUHO. Also, these indices remained associated with MUHO after adjustment for age, gender, visceral fat area and HOMA-IR (Table 3).

Discussion

The gold standard of modern medicine is precise medicine. As a rule of thumb mainly in clinical practice, detailed phenotyping is the first stage of diagnosis. Considering these remarks, it is important to update the status of biomarkers/indices used in obesity and metabolic syndrome, which can help clinicians to decipher the above conditions. In this sense, here, we aimed to provide more details on the relationship between different indices and MUHO, for a detailed phenotyping analysis and individualization of the therapeutic approach.

In our research, we identified a high frequency of MUHO and metabolic syndrome (72%). This frequency is higher than the prevalence reported in the PREDATORR study, where the age and sex-adjusted overall prevalence of MUHO was 3.9% and that of metabolic syndrome was 38.5% [20]. This difference can be explained by the characteristics of the samples included – a sample representative of the Romanian population in the PREDATORR study and a selected

Table 1: Clinical, metabolic, and anthropometric characteristics.

	Total n = 163	MHO n = 45	MUHO n = 118	p value
Women, n (%)	83 (50.9%)	31 (68.9%)	52 (44.1%)	0.005
Age	38.1 ± 11.2	34.5 ± 10.5	39.5 ± 11.3	0.013
Weight, kg	110.1 ± 19.5	103.0 ± 18.4	112.9 ± 19.4	0.004
BMI (kg/m ²)	37.2 ± 5.5	35.9 ± 5.1	37.8 ± 5.7	0.052
Waist circumference (cm)	115.3 ± 13.4	109.0 ± 10.6	117.8 ± 13.7	<0.001
WHR	1.0 (1.0; 1.1)	1.0 (1.0; 1.0)	1.0 (1.0; 1.1)	0.015
Medical history				
CVD (%)	46 (28.2%)	10 (22.2%)	36 (30.5%)	0.335
DLP (%)	51 (31.3%)	5 (11.1%)	46 (39.0%)	0.001
HTW (%)	76 (46.6%)	1 (2.2%)	75 (63.6%)	<0.001
Metabolic syndrome	118 (72.4%)	0 (0.0%)	118 (100.0%)	<0.001
Total cholesterol (mg/dL)	189.0 ± 40.8	183.0 ± 31.6	191.4 ± 43.7	0.182
HDL cholesterol (mg/dL)	43.3 ± 14.7	50.2 ± 12.6	40.8 ± 14.7	<0.001
LDL cholesterol (mg/dL)	112.8 ± 36.9	110.8 ± 28.6	113.6 ± 39.7	0.629
Triglycerides (mg/dL)	146.0 (102.0; 203.0)	102.0 (81.9; 127.5)	170.0 (115.0; 239.0)	<0.001
Creatinine (mg/dL)	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.154
TGO (U/L)	26.7 (21.8; 35.0)	27.0 (19.9; 33.0)	26.4 (22.0; 36.0)	1.000
TGP (U/L)	32.0 (22.8; 50.9)	36.0 (19.8; 50.8)	36.0 (19.8; 50.8)	0.720
Glycemia (mg/dL)	99.5 ± 15.5	89.8 ± 12.6	103.3 ± 15.0	<0.001
Insulinemia (μU/mL)	18.4 (12.0; 28.2)	14.9 (10.6; 26.6)	19.9 (12.5; 28.5)	0.016
HOMA-IR	4.6 (2.7; 7.0)	3.2 (2.4; 6.0)	5.1 (3.2; 7.3)	0.016
Insulin resistance	129 (79.1%)	31 (68.9%)	98 (83.1%)	0.054
VFA (cm ²)	168.0 ± 46.6	150.4 ± 41.5	174.9 ± 46.9	0.002
SMM (kg)	36.3 ± 7.7	33.2 ± 6.8	37.5 ± 7.7	0.001
BFM (kg)	45.1 ± 13.2	43.7 ± 12.8	45.8 ± 13.4	0.360

Note: BMI – body mass index, VFA – visceral fat area, SMM – skeletal muscle mass, BFM – body fat mass, WHR – waist-hip ratio, HOMA-IR – homeostasis model assessment-insulin resistance, LAP – lipid accumulation product.

Table 2: Atherogenic indices in the whole sample and in patients with and without metabolically unhealthy obesity.

	Total n = 163	MHO n = 45	MUHO n = 118	p value
Non-HDL cholesterol	145.6 ± 40.2	132.8 ± 29.6	150.0 ± 42.7	0.003
LAP	82.2 (57.7; 142.2)	55.4 (43.5; 69.1)	106.5 (68.7; 162.1)	<0.001
Atherogenic index	3.5 (2.4; 4.8)	2.4 (2.1; 3.4)	3.9 (2.7; 5.2)	<0.001
Total cholesterol/HDL cholesterol	4.4 (3.4; 5.8)	3.4 (3.1; 4.4)	4.9 (3.7; 6.2)	<0.001
LDL cholesterol/HDL cholesterol	2.5 (1.8; 3.7)	2.0 (1.7; 3.0)	2.9 (2.1; 3.9)	0.001
Non-HDL cholesterol/HDL cholesterol	3.4 (2.3; 4.8)	2.4 (2.1; 3.4)	3.7 (2.7; 4.9)	<0.001

Note: LAP – lipid accumulation product.

Table 3: Unadjusted and adjusted OR for atherogenic indices, lipid profile and body composition parameters as predictors of metabolically unhealthy obesity.

	Unadjusted OR (95% CI)	Adjusted* OR (95% CI)
Non-HDL cholesterol	1.012 (1.003; 1.022)	1.012 (1.001; 1.022)
LAP	1.036 (1.021; 1.052)	1.036 (1.019; 1.052)
Atherogenic index	1.848 (1.370; 2.492)	1.837 (1.337; 2.525)
Total cholesterol/HDL cholesterol	1.848 (1.370; 2.492)	1.837 (1.337; 2.525)
LDL cholesterol/HDL cholesterol	1.549 (1.112; 2.139)	1.611 (1.132; 2.292)
Non-HDL cholesterol/HDL cholesterol	1.848 (1.370; 2.492)	1.837 (1.337; 2.525)

Note: *Adjusted for age, gender, visceral fat area, HOMA-IR. LAP – lipid accumulation product.

sample consisting of patients presenting for obesity counseling in our study. Also, in the PREDA-TORR study, prevalence was adjusted for age and sex, while in our sample we provide the crude prevalence.

Our study showed that MUHO participants were older and had a higher waist circumference and BMI compared to MHO subjects. This is in line with the observations in the Malmö diet cancer study, in which Korduner et al. found that MHO participants were younger, more likely to be men, had a lower BMI, and a lower waist and hip circumference [21]. It is well-known that waist circumference is a marker for cardiovascular risk and also for mortality and morbidity. A systematic review of >680,000 participants, with a follow-up of 24 years demonstrated that waist circumference was associated with increased all-cause mortality [6, 7]. In a consensus statement, the association between abdominal obesity and mortality of any cause, after adjustment according to BMI is highlighted [6]. A 10% larger abdominal circumference increases the risk of mortality 1.48 times. Nelms et al. demonstrated in a study with over 43,000 participants that waist circumference was related to non-fatal, fatal CVD and all-cause mortality in men, and only to fatal CVD and all-cause mortality in women, after adjustment for age and smoking status [8].

The main parameters that could be considered to help the clinician are hypertensive waist, atherogenic index and lipid accumulation product. In the present study, 46.6% of patients had a hypertensive waist. This is in line with a

previous study conducted in the Romanian population, which included 1294 randomly selected persons and reported a 43.3% prevalence of hypertensive waist [17]. Since 2005, when LAP was introduced as a better atherogenic index than BMI in predicting CVD, numerous studies have shown that over 10 years of follow-up, LAP can be a useful index. In the ATTICA study, the mean LAP value at baseline was 69% higher in participants who developed a CV event [18]. LAP has a higher predictive accuracy for metabolic syndrome than other indices, as mentioned in a study on 15,490 participants [22]. Regarding the best independent predictor of hypertension, this was LAP (OR: 2.461 [95% CI: 2.277–2.660]) in a National Health Examination Survey study of 2009 (NHES-IV) [23]. In identifying the MHO phenotype, blood pressure measurement and LAP estimation are described as a useful method in postmenopausal women [24]. In our study, LAP and AI were significantly higher in patients with MUHO. Abolnezhadian F. et al. demonstrated that cardiovascular indices, including AI, were significantly higher in MUHO and metabolically unhealthy normal weight phenotype as compared to MHO and healthy normal weight phenotype [3]. AI is associated with a higher risk of subclinical coronary artery disease, beyond traditional risk factors [25]. Depending on the presence of metabolic factors, AI increases progressively and also, a score ≥ 2 is less common in metabolically normal phenotype than in metabolically unhealthy phenotype [26]. As for the lipid profile, we found that patients with MUHO

had lower HDL cholesterol levels and higher triglycerides compared to MHO. No difference between groups was observed for total cholesterol and LDL cholesterol. Similar results were reported by Telle-Hansen et al., excepting the LDL cholesterol levels, which were found to be correlated with MUHO [27]. Regarding LDL cholesterol levels, the results of our study are consistent with the results of Catoi et al., which showed no statistically significant differences between MHO and MUHO [28]. Also, in our study, total cholesterol/HDL cholesterol ratio, LDL cholesterol/HDL cholesterol ratio, and non-HDL cholesterol/HDL cholesterol ratio were higher in the MUHO phenotype than in MHO. This is important as these indices have been associated with the risk of high carotid intima-media thickness in a prospective study of 13,612 participants followed for a two-year period. Also, there is a 31% reduction in the risk of major cardiovascular events with every 1% reduction in LDL-C/HDL-C ratio [29]. Another finding of our study is that all atherogenic indices were associated with the presence of MUHO even after adjustment for age, gender, visceral fat area and HOMA-IR, and therefore the risk of CVD is higher for these patients. The MUHO phenotype is characterized by ectopic fat and impaired adipose tissue function, leading to insulin resistance. The adipokine secretion pattern and pro-inflammatory status may also contribute to the MUHO phenotype [11]. Insulin resistance could explain many of the risks associated with metabolic syndrome, MUHO respectively (depending on the definition), and these data may help clinicians focus on the prevention of cardiometabolic diseases.

Although our goal was achieved, some limitations of the study should be mentioned. First, the sample included in the analysis was limited. Also, we collected data retrospectively and we included patients presenting for health evaluation regarding obesity and nutrition counseling.

Conclusions

In conclusion, in this study we described in patients with MUHO several clinical indices/parameters accessible in current practice,

which can help clinicians to identify the MUHO phenotype, focus on cardiovascular risk, and to implement the required preventive measures. Knowledge of metabolic health can help the practitioner to take a stand and inform the patient about the benefits of lifestyle optimization to reduce cardiovascular risk. The modern approach means precise medicine based on detailed phenotyping. Even if precise medicine is a combination between genotyping and phenotyping, in medical practice, simple and useful parameters are needed to assess cardiovascular risk. The first step towards precise medicine within diagnosis should include accurate phenotyping for individualization of the therapeutic approach.

Acknowledgements

This study was possible with the financial support of “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania through the internal research grant no. 7690/82/15.04.2016 and 5200/71/1.03.2017.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

The relevance of adiponectin in diabetic patients during the dental implant

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Received: 18 May 2021 / Accepted: 10 June 2021

Abstract

Background and Aims: Adiponectin is a peptide hormone secreted by adipocytes and plays a protective role against the development of insulin resistance. Diabetes mellitus, or uncontrolled glycemic level, is one the factors for the failure of dental implant. The study aimed to show the relevance of adiponectin in diabetic patients during dental implants. **Materials and Methods:** The study was conducted on 152 patients. Out of these, 94 were males and 58 were females, 71 were diabetics following treatment anti-diabetic drugs or on insulin therapy, or on both and 81 were non-diabetics. The anthropometric measurements and the blood pressure were noted and the glycemic and the adiponectin levels were assessed before and after the implant. **Results:** The study revealed that adiponectin was inversely correlated and it decreased with the increase in body mass index. In comparing the diabetic group with the non-diabetic one, before and after the implant, the average adiponectin was significantly higher in the non-diabetic group (5.03 vs. 2.16, $p < 0.001$, respectively 5.24 vs. 2.24, $p < 0.001$). **Conclusions:** The study concludes that adiponectin is one of the potential biomarkers for the early detection of the development of diabetes and metabolic syndrome and that its low levels might lead to the failure of the dental implant.

Keywords: adiponectin, dental implant, diabetes mellitus

Introduction

The adipose tissue is the biggest endocrine organ that secretes cytokines, mainly adipokines, which regulates insulin sensitivity along with inflammatory process [1]. Adiponectin is a peptide hormone secreted by adipocytes and plays a protective role against development of insulin resistance [2], having in the same time anti-inflammatory effects [3]. The physiological values are considered 5–30 $\mu\text{g/mL}$ [4], but they may vary according to genders, higher in women than in men [5], and on ethnic, Caucasians have higher value than Indo-Asians [6]. The decrease in the levels of adipokines is one of the key factors

giving rise to complications of obesity, such as diabetes type 2 and cardiovascular diseases [7].

Diabetes mellitus is a metabolic disorder represented by chronic hyperglycemia and one of the leading causes of mortality worldwide. Type 2 diabetes is defined as lack of production of insulin, or insulin resistance, and represents around 95% of all diabetes.

The most common oral complication of diabetes mellitus includes gingivitis and periodontitis, leading to premature tooth loss [8], poor wound healing [9] and impaired response to infection, that affect the patients' quality of life. Diabetes mellitus, or uncontrolled glycemic levels, is one the factors for the failure of dental implant [10]. Dental implantation is a



widely done surgical procedure for dental restoration. The implant mimics the root of a tooth in function and fuses to bone by osseointegration. Patients with poorly controlled diabetes seem to have delayed osseointegration following implantation. Good glycemic control improves osseointegration and implant survival. Dental rehabilitation improves nutrition and the metabolic control of a patient and allows to lead a healthy and quality life.

Material and Methods

The study was conducted on 152 patients going for the dental transplant. Out of these, 94 were males and 58 were females, 71 patients were having diabetes type 2, being treated with anti-diabetic drugs or on insulin therapy, or on both, whereas 81 patients were without diabetes. We included the patients above 18 years old and without any malignant condition. Blood samples were taken to evaluate the metabolic profile and adiponectin levels, after overnight fast, between 07 am and 08 am, before and after the dental implant process. The adiponectin testing was done by ELISA kits according to manufacturer’s guidelines.

Results

The results of our study, according to the sociodemographic, clinical and para-clinical parameters of non-diabetic and diabetic groups, are shown in Table 1.

In our study, patients with diabetes and non-diabetes had body mass index ranging from 20.9–38.8 kg/m² and 20.1–39.9 kg/m². The study revealed that according to body mass index, adiponectin in inversely correlated and decreases with the increase in body mass index (Figure 1).

Patients with diabetes showed low levels of adiponectin as compared to non-diabetic patients. The range of glycemia in the diabetic group was 88–204 mg/dL and 99–200 mg/dL (before and after implant), while in the non-diabetic was 71–119 mg/dL and 76–116 mg/dL (before and after implant). In both groups the mean value of adiponectin increased insignificantly before and after implant in diabetic group (2.16 vs. 2.25, p = 0.790, respectively 5.03 vs. 5.24, p = 0.143). While comparing the diabetic group with the non-diabetic one, before and after implant, the average adiponectin was significantly higher in the non-diabetic group (5.03 vs. 2.16, p < 0.001, respectively 5.24 vs. 2.24, p < 0.001) (Figure 2).

Discussion

In this study, we have shown the adiponectin concentrations in diabetic and non-diabetic patients before and after dental implants. The levels of adiponectin were low in diabetic than non-diabetic group. After dental implant and improved glycemic levels, a mild increase in adiponectin levels were observed in diabetic patients.

Adiponectin has been emerging as an antidiabetic and as an antiatherogenic agent. It’s

Table 1:

	Non-diabetic group	Diabetic group	p value
Gender (M/F)	51/30	43/28	p = 0.762
Age range (years)	32–70	44–81	p < 0.001
MD ± DS	52.35 ± 9.41	59.11 ± 6.48	
Average	52.11	59.11	
BMI range (kg/m²)	20.1–39.9	20.9–38.8	
Adiponectin range (T0/T1)	5.03–24.70/5.24–28.76	2.16–16.30/2.25–13.20	p = 0.143/p = 0.790
MD ± DS	12.92 ± 4.04/13.86 ± 4.09	6.52 ± 2.31/6.62 ± 2.15	

T0 – before implant, T1 – after implant.

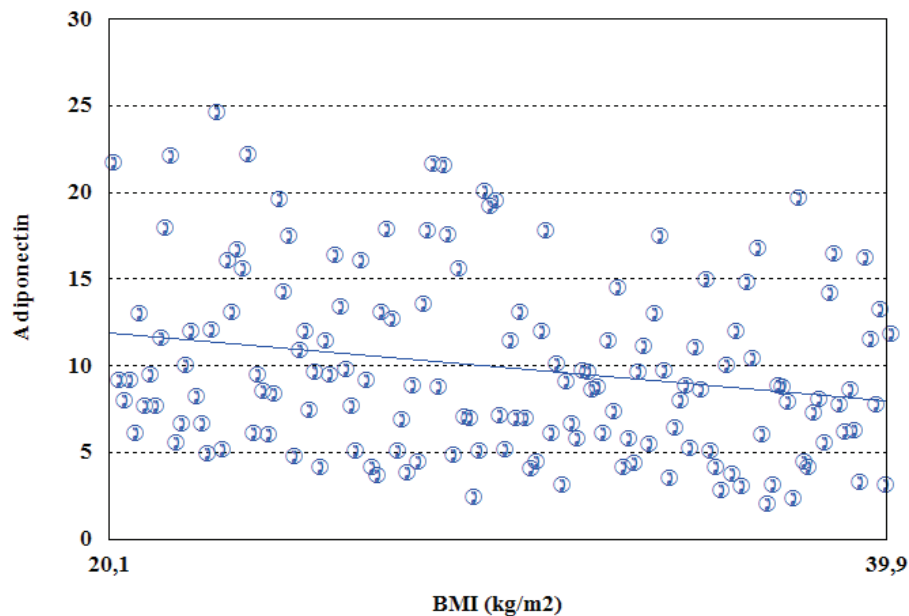


Figure 1: Adiponectin values and the body mass index.

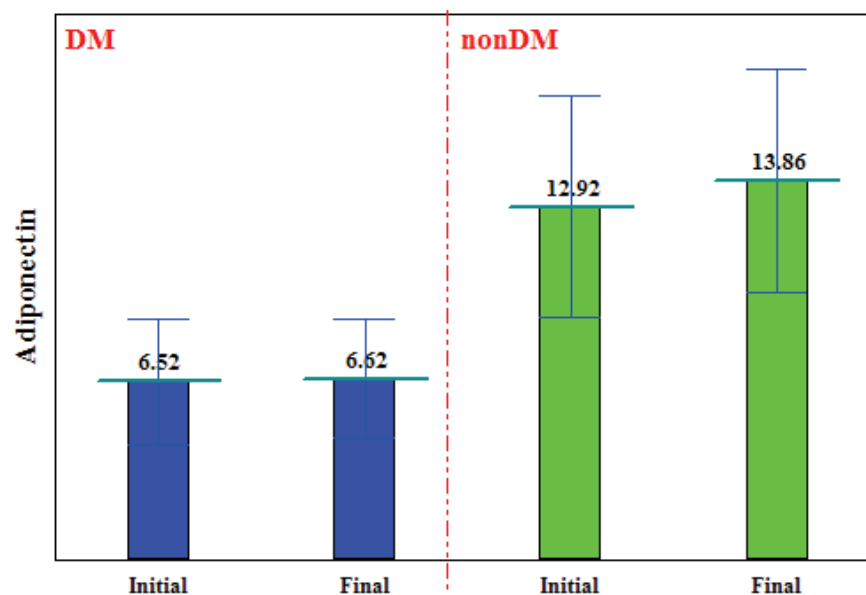


Figure 2: The adiponectin values before (initial) and after (final) dental implant.

circulation level is influenced by different factors such as age, gender, race, smoking and other medical problems [11].

Obesity is becoming a serious worldwide health issue and one of the most important factors for the development of metabolic and cardiovascular diseases. The abnormal deposition of fat leads to the decrease production of adiponectin [12]. Obesity, or chronic inflammatory state, further advances to the development of insulin

resistance and diabetes mellitus [13]. Previous studies have demonstrated that adiponectin levels decline in patients with metabolic syndrome, diabetes, obesity and in hypertension [14]. The same thing has been observed in our study, the levels of adiponectin in the diabetics compared to the non-diabetic group were low.

A study done by Yang et al. [15] has also demonstrated low levels of adiponectin in obese patients.

In both groups, the diabetic and the non-diabetic one, no significant increase of adiponectin was noticed, before or after dental implant, but higher values were observed in the non-diabetic group. Our study also showed that adiponectin levels are inversely related to diabetes mellitus type 2 patients.

It is a well-known fact that adiponectin reduces insulin resistance and affects bone metabolism. Adiponectin was demonstrated to play a crucial role in osteoblast differentiation and bone formation in cultured osteoblastic cells. Adiponectin enhanced bone morphogenetic protein 2 (BMP-2) and Runx2 expression and induced the commitment of mesenchymal stem cells into an osteoblast lineage, as well as the differentiation of osteoblasts [16]. Adiponectin is known for its role in enhancing chondrocyte proliferation and matrix mineralization [17], but still the relationship between adiponectin and body mineral density is controversial [18].

The studies suggest that adipocytes, as well as bone cells, contribute to the production and high concentration of adiponectin, found in bone marrow [19, 20]. Adiponectin stimulates the proliferation and mineralization of human osteoblasts through autocrine and/or paracrine and endocrine pathways [21].

It is also reported that the adiponectin receptor is expressed in not only osteoblasts, but also osteoclastic cells, and that adiponectin directly inhibits osteoclastogenesis and bone resorption. Adiponectin significantly inhibits M-CSF- and RANKL-induced osteoclast differentiation and function, and inhibits osteoprotegerin production in osteoblasts [22].

Y. Bai et al., in 2011, demonstrated that systemic adiponectin infusion (2.5 µg of mouse recombinant globular adiponectin per day) could significantly accelerate osteogenesis peri-implant [23].

This can improve the success ratio of dental implants in type 2 diabetic patients, by systemically infused or locally used adiponectin, and could also accelerate the osseointegration of dental implants.

Diabetes mellitus leads to the reduction in bone mineral density [24], which could be reversed by the effect of adiponectin on bone

metabolism. All these observations suggested that adiponectin could improve osseointegration of dental implants in diabetic patients, by regulating bone remodeling when applied systemically infused or locally used.

Conclusions

Adiponectin is one of the potential biomarkers for the early detection of the development of diabetes and metabolic syndrome. Adiponectin is known as anti-inflammatory and anti-atherogenic, but besides this it also improves insulin sensitivity and glucose metabolism.

Finally, the low levels of serum adiponectin, as observed in obesity and diabetic patients, may jeopardize periodontal healing and might lead to the failure of dental implant.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

Retinopathy and chronic kidney disease in type 2 diabetes mellitus

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Received: 16 May 2021 / Accepted: 10 June 2021

Abstract

Aims: To evaluate the prevalence of diabetic retinopathy (DR) and chronic kidney disease (CKD) and also the association between DR and CKD and their association with cardiometabolic and sociodemographic factors, in patients with type 2 diabetes. **Materials and Methods:** We studied medical records of 443 consecutive hospitalized adult patients with type 2 diabetes. Diabetic chronic complications, insulin secretion, diabetes therapy, control and duration, cardiometabolic and sociodemographic data were assessed. **Results:** The current study indicates that the overall prevalence of DR was 63.4%. Of these, 31.6% had non-proliferative DR and 31.8% had proliferative DR. The overall prevalence of CKD was 51.7%, of which 26.2% had only eGFR < 60 mL/min/1.73m², 39.5% had only albuminuria, and 11.8% (0.14–0.74%) had both. In 28.9% of patients, DR and CKD were associated, and only 12.7% of patients had neither DR nor CKD. The simultaneous presence of DR and CKD was significantly associated with diabetic peripheral neuropathy, peripheral artery disease and also with long lasting diabetes. About 2.4% of patients with DR associated with CKD were newly diagnosed with diabetes. Patients with DR associated with CKD presented significantly higher BMI and waist compared with patients with CKD alone and with patients without DR or CKD and higher uric acid levels compared with patients with DR alone and with patients without DR or CKD. **Conclusions:** The results of this study indicate a high prevalence of DR and CKD both isolated and associated in hospitalized type 2 diabetic patients, providing data on their prognosis and association with several cardiometabolic risk factors.

Keywords: cardiometabolic factors, chronic kidney disease, diabetic retinopathy, type 2 diabetes

Background and Aims

Diabetes represent one of the most important and frequent metabolic disease worldwide and is expected to increase in prevalence in conjunction with rising rates of obesity, population growth and ageing [1–3]. Moreover, the Global Burden of Disease Study 2010 indicated that between 1990 and 2010, diabetes was the non-communicable disease with the fastest increasing contribution to the burden of lost disability-adjusted life-years [4]. The prevention

and control of diabetic chronic complications is important because the morbidity and mortality related with diabetes impose a global burden on socioeconomic development [5].

Diabetic nephropathy is a well-described diabetic complication and the leading cause of end stage renal disease and diabetic retinopathy (DR) is a highly specific visual complication of diabetes and the leading cause of blindness [6–8].

Despite attempts to link the two most important microvascular complications of diabetes, retinopathy and nephropathy, the



relationship has not so far been clearly described. Diabetes duration and glycemic, blood pressure, and lipid control have been shown to be consistently correlated with microvascular complications of diabetes, but to date, the relationship of one diabetic microvascular complication to another has not been fully clarified. In addition to sharing risk factors, such as hyperglycemia and hypertension, DR and chronic kidney disease (CKD) are reflected in the clinical manifestations of similar microvascular lesions in the glomerular and retinal vessels [8, 9]. Several studies have documented the association between DR and albuminuria in people with type 2 diabetes mellitus (T2DM) [10, 11], but on the other hand, DR has been shown to be a risk factor for microalbuminuria and macroalbuminuria [12].

However, the currently literature data suggest that the presence of a pre-existing retinopathy or nephropathy may contribute to the development of another [13, 14].

The main objective of this research was to evaluate the prevalence of DR and CKD and also the association between DR and CKD in patients with T2DM. The current study was designed also with the aim of evaluating the possible association of DR and CKD with cardiometabolic and sociodemographic factors in patients with T2DM.

Materials and Methods

The present study is a cross-sectional study conducted by analyzing the medical records of unselected patients with T2DM hospitalized in the Emergency Clinical Hospital Craiova, Department of Diabetes, Nutrition and Metabolic Diseases, during 1-year period.

In our study, we analyzed medical records from the last available hospitalization during which clinical evaluation and all procedures were performed in order to assess diabetes control, diabetic chronic complications, cardiometabolic factors and other comorbidities.

The survey was conducted according to the International Conference on Harmonization—Good Clinical Practice Guidelines standards and World Medical Association

Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants (Seoul, 2008) and being a retrospective analysis of medical data routinely recorded in our hospital database in compliance with European Union General Data Protection Regulation, no further approval of an ethics committee is required. All patients admitted to the hospital signed informed consent for the use of their medical data for research purposes.

Clinical and biological data

Information regarding sociodemographic (age, gender), anamnestic data (maximum reported weight, DM duration, type and doses of DM antidiabetic therapy, diabetic chronic micro- and macrovascular complications, personal medical history of hypertension, dyslipidemia and obesity, anti-hypertensive, or lipid lowering therapy), clinical and biological data were collected from the electronic medical record.

Clinical evaluation included assessment of anthropometric parameters: height, weight, and waist circumference, body mass index (BMI), systolic (SBP) and diastolic blood pressure (DBP). Subjects with a BMI ≥ 25 kg/m² were considered as having overweight/obesity. Abdominal obesity was defined as a waist circumference ≥ 80 cm in women or ≥ 94 cm in men. Hypertension was defined as SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg and/or antihypertensive therapy, or a personal history of hypertension.

The following biochemical data were analyzed from the electronic medical records: glycated hemoglobin (HbA_{1c}), fasting plasma glucose (FPG), C-peptide, total cholesterol (TC), triglycerides, HDL cholesterol (HDL-C), creatinine, GOT, GPT, GGT, uric acid, albuminuria. LDL cholesterol (LDL-C) were calculated using the Friedewald formula if total triglycerides levels were < 400 mg/dL.

Diagnosis of hypo-HDL cholesterolaemia was considered when HDL-C level < 40 mg/dL in men or < 50 mg/dL in women or drug treatment for low HDL-C and hypertriglyceridemia when triglycerides level ≥ 150 mg/dL or drug treatment for hypertriglyceridemia.

Hypercholesterolemia was defined as TC $\geq$ 200 mg/dL and/or statin therapy and hyper-LDL cholesterolaemia was defined as LDL-C $\geq$ 100 mg/dL and/or statin therapy.

Poor controlled diabetes was considered when HbA_{1c} > 7%.

Hyperuricemia was considered when uric acid levels were $\geq$ 6 mg/dL in women or $\geq$ 7 mg/dL in men. Normal reference interval for C-peptide was considered 0.8–3.8 nmol/L.

Diabetic chronic complications

Diagnosis of macrovascular complications (ischemic heart disease, stroke, peripheral arterial disease) was based either on clinical data (peripheral arterial pulse, lower limb non-traumatic amputation, motor deficits, personal history of angioplasty or arterial by pass) or para-clinical data (EKG, CT, MRI, Doppler ultrasounds, angiography) from medical records.

Diagnosis of DR was based on eye fundus examination results registered in the electronic medical records. The findings from eye fundus examination were used to classify eyes as having one of two phases of DR: non-proliferative DR and proliferative DR. Diabetic macular edema was an additional complication that was recorded separately from the retinopathy stages. Laser therapy was also recorded.

Diagnosis of diabetic peripheral neuropathy was based on Toronto score which was evaluated using signs, symptoms and neuropathy tests results recorded in the medical charts. Toronto score higher than 6 was considered positive.

CKD was defined based on estimated glomerular filtration rate (eGFR) and albumin excretion rate, according to Kidney Disease Improving Global Outcomes 2012 guidelines. CKD was defined as eGFR < 60 mL/min/1.73 m² (CKD-EPI equation) and/or albuminuria.

Statistical analysis

Continuous variables were expressed as mean and standard deviation. The categorical variables were expressed as a percentage. The

differences in sociodemographic and clinical-biological parameters according to the presence of DR and CKD were tested using parametric tests (t-test, ANOVA) or non-parametric Mann-Whitney test for the continuous variables and Chi-square for the categorical variables. A $p < 0.05$ (two-tailed) was considered statistically significant. The statistical analysis was performed using the SPSS v19.0 software.

Results

The current analysis includes 443 subjects (230 female/213 male, 61.6 $\pm$ 10.9 years, 3.8% in the 20–39 age group, 38.4% in the age group 40–59 years, 54.6% in the age group of 60–79 years and 3.8% in the >80 years age group) admitted in the Clinic of Diabetes, Nutrition and Metabolic Disease from Emergency Clinical Hospital Craiova.

Prevalence of diabetic retinopathy

The overall prevalence of DR was 63.4%. Of these, 31.6% had non-proliferative DR and 31.8% had proliferative DR (Figure 1). There were no significant age and gender differences in the prevalence of DR (Table 1).

Laser therapy was performed in 44% of patients with proliferative DR and in 1.4% of patients with non-proliferative DR.

24.1% of the patients with proliferative DR had maculopathy and only 6.4% of the patients with non-proliferative DR had maculopathy.

Vitreous hemorrhage was found in 5.7% of the patients with proliferative DR.

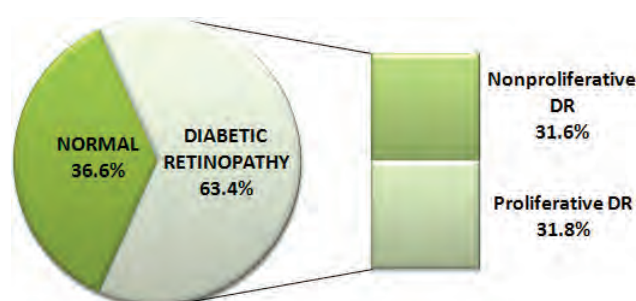


Figure 1: Prevalence of diabetic retinopathy. DR, diabetic retinopathy.

Table 1: Prevalence of diabetic retinopathy by gender and age groups.

	Age (years)				Overall
	20–39	40–59	60–79	>80	
Total population					
DR present	64.3	64.7	64.5	35.3	63.4
Non-proliferative DR	42.9	30.6	32.2	23.5	31.6
Proliferative DR	21.4	34.1	32.2	11.8	31.8
Men					
DR present	60.0	65.1	60.6	50.0	62.0
Non-proliferative DR	30.0	24.4	28.4	25.0	26.8
Proliferative DR	30.0	40.7	32.1	25.0	35.2
Women					
DR present	75.0	64.3	67.7	22.2	64.8
Non-proliferative DR	75.0	36.9	35.3	22.2	36.1
Proliferative DR	0.0	27.4	32.3	0.0	28.7

Prevalence of chronic kidney disease

The overall prevalence of CKD was 51.7%, of which 11.8% had eGFR < 60 mL/min/1.73m² associated with albuminuria (Figure 2). Analysis of the eGFR categories distribution indicated that 26.2% of the participants had stage 3–5 CKD (Figure 2). Albuminuria was detected in 39.5% of the analyzed subjects (Figure 2, Table 2).

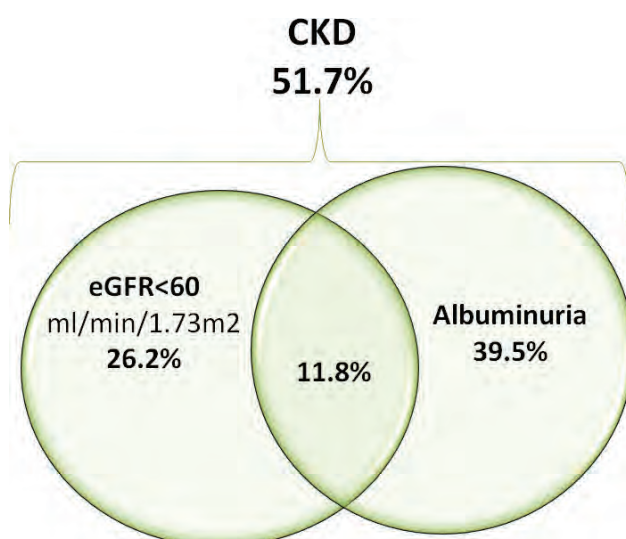


Figure 2: Prevalence of chronic kidney, albuminuria and eGFR < 60 mL/min/1.73m². CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

The prevalence of CKD increased significantly with age ($p = 0.03$), the highest prevalence being in the 80+ age group (Table 2). Regarding the gender distribution of CKD prevalence there were no significant differences (Table 2).

The distribution of eGFR categories by gender, age groups and overall are presented in Table 2. Stage 3–5 CKD predominated in patients over 60 years of age, overall but also in both genders, except CKD stage G5, which predominated in males in the 40–59 age group (Table 2). Albuminuria was predominant in patients over 80 years of age for both sexes (Table 2).

Prevalence of diabetic retinopathy associated with chronic kidney disease

Isolated DR was detected in 35.6% of patients, while CKD alone was diagnosed in 22.9% of patients (Figure 3). Only 12.7% of patients had neither DR nor CKD.

Diabetic retinopathy and CKD were significantly associated in analyzed T2DM patients ($p < 0.001$). In 28.9% of patients, DR and CKD were associated (Figure 3).

Patients with stage G3a and G3b CKD had predominantly normal eye fundus examination, suggesting that not only diabetes contributed to

Table 2: Prevalence of chronic kidney disease by gender and age groups.

	Age (years)				Overall
	20–39	40–59	60–79	>80	
Total population					
CKD present	35.7	45.2	56.0	70.6	51.7
G1 (eGFR ≥90 mL/min/1.73 m²)	92.9	56.9	15.0	5.9	33.4
G2 (eGFR: 60–89 mL/min/1.73 m²)	7.1	30.5	46.8	76.5	40.4
G3a (eGFR: 45–59 mL/min/1.73 m²)	0.0	5.4	18.9	17.6	13.0
G3b (eGFR: 30–44 mL/min/1.73 m²)	0.0	4.8	13.3	0.0	9.0
G4 (eGFR: 15–29 mL/min/1.73 m²)	0.0	0.6	4.3	0.0	2.6
G5 (eGFR <15 mL/min/1.73 m²)	0.0	1.8	1.7	0.0	1.6
Albuminuria	35.7	41.0	37.0	64.7	39.5
Men					
CKD present	50.0	49.4	59.8	75.0	55.7
G1 (eGFR ≥90 mL/min/1.73 m²)	90.0	65.9	17.8	0.0	40.0
G2 (eGFR: 60–89 mL/min/1.73m²)	10.0	17.6	40.2	75.0	31.0
G3a (eGFR: 45–59 mL/min/1.73 m²)	0.0	8.2	19.6	25.0	14.3
G3b (eGFR: 30–44 mL/min/1.73 m²)	0.0	4.7	17.8	0.0	11.0
G4 (eGFR: 15–29 mL/min/1.73 m²)	0.0	1.2	2.8	0.0	1.9
G5 (eGFR <15 mL/min/1.73 m²)	0.0	2.4	1.9	0.0	1.9
Albuminuria	50.0	42.4	41.9	62.5	43.3
Women					
CKD present	0.0	41.0	52.8	66.7	48.0
G1 (eGFR≥90 mL/min/1.73 m²)	100.0	47.6	12.7	11.1	27.1
G2 (eGFR: 60–89 mL/min/1.73 m²)	0.0	43.9	52.4	77.8	49.3
G3a (eGFR: 45–59 mL/min/1.73 m²)	0.0	2.4	18.3	11.1	11.8
G3b (eGFR: 30–44 mL/min/1.73 m²)	0.0	4.9	9.5	0.0	7.2
G4 (eGFR: 15–29 mL/min/1.73 m²)	0.0	0.0	5.6	0.0	3.2
G5 (eGFR <15 mL/min/1.73m²)	0.0	1.2	1.6	0.0	1.4
Albuminuria	0.0	39.5	33.1	66.7	36.1

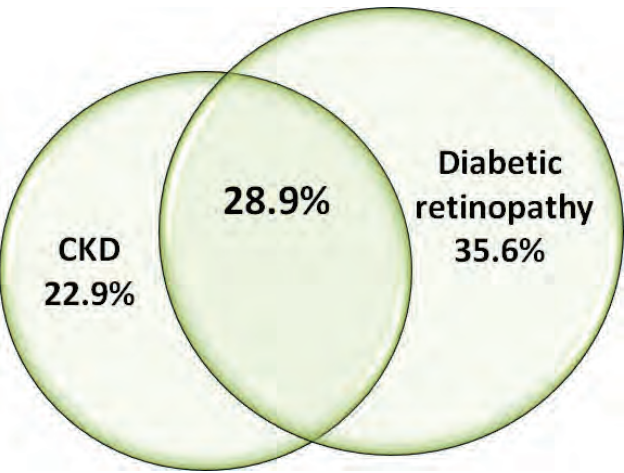


Figure 3: Association of chronic kidney disease with DR. CKD, chronic kidney disease.

CKD pathogeny, but also other potential causes such as hypertension, chronic pyelonephritis, hyperuricemia, etc. (Table 3).

In advanced stages of CKD (G4-G5), proliferative DR was predominantly detected, indicating diabetes as a leading cause of CKD progression (Table 3).

Analysis of factors associated with diabetic retinopathy and/or chronic kidney disease in patients with type 2 diabetes

The simultaneous presence of DR and CKD was significantly associated with

Table 3: Association of eGFR categories with diabetic retinopathy stages.

		Diabetic retinopathy (%)		
		Normal	Non-proliferative DR	Proliferative DR
eGFR categories (%)	G1	11.4	11.8	10.2
	G2	12.8	13.9	13.7
	G3a	6.3	3.0	3.7
	G3b	4.2	1.9	3.0
	G4	0.5	0.9	1.2
	G5	0.5	0.5	0.7

obesity, abdominal obesity, diabetic peripheral neuropathy, peripheral artery disease and also with hyperuricemia (Table 4).

Most of the patients with DR associated with CKD had long lasting diabetes with duration higher than 10 years (Table 4). About 2.4% of patients with DR associated with CKD were newly diagnosed with DM (Table 4).

Patients with DR associated with CKD presented significantly higher BMI and waist compared with patients with CKD alone and with patients without DR or CKD (Table 5).

The DM duration was significantly higher in patients with DR associated with CKD compared with patients with DR alone or CKD alone and also with patients without DR or CKD (Table 5).

Patients with DR associated with CKD presented significantly lower eGFR and higher uric acid levels compared with patients with DR alone and with patients without DR or CKD (Table 5).

Discussion

The current study indicates that the overall prevalence of DR was 63.4%, with similar distribution of non-proliferative and proliferative DR and the overall prevalence of CKD was 51.7%, of which 26.2% had stage 3–5 CKD and 11.8% had eGFR < 60 mL/min/1.73m² associated with albuminuria. DR and CKD were associated in 28.9% of patients.

These findings are similar to other studies, although some reported that non-proliferative

DR is more common. Thus, a study performed in Korea indicated that the overall prevalence of DR was 67.76%, from these 39.3% had non-proliferative DR and 28.4% had proliferative DR [15]. Liu Y. et al. reports that the prevalence of DR was 34.08% among diabetic patients from Mainland China, non-proliferative DR being more frequent [16]. Several studies indicated that the increased duration of DM is a risk factor for developing DR [17, 18].

Another finding of the current study was the high prevalence of CKD among hospitalized T2DM patients, results which are consistent with those reported by Thomas et al. in a study performed in Australia (23.1% of T2DM patients from primary care had eGFR < 60 mL/min/1.73 m² and 34.6% had albuminuria) and by New J. P., et al. in a study performed in the United Kingdom (eGFR < 60 mL/min/1.73 m² was detected in 31% of diabetic patients and albuminuria in 37% of these patients) [19, 20]. However, the prevalence of stage 3–5 CKD in T2DM was lower than that reported by Vinagre et al. (20% for renal impairment and 16.7% for albuminuria) and by Rodriguez-Poncelas A. et al. in a sample of 1145 T2DM patients treated in primary care consults in Spain (18% had a eGFR < 60 mL/min/1.73 m² and 15.4% had albuminuria) [21, 22].

Current study indicated that in 28.9% of patients, DR and CKD were associated, and only 12.7% of patients had neither DR, nor CKD. These findings are different from those reported by Penno G. et al. in the large cohort of the RIACE Italian multicenter study, which indicate that the majority of subjects (51.87%) had neither DR nor CKD, and an association between DR and CKD

Table 4: Factors associated with diabetic retinopathy and/or chronic kidney disease.

Variables (%)		Without DR or CKD	DR alone	CKD associate with DR	CKD alone	p value
Number of participants		55	154	125	99	
Body mass index (kg/m ²)	Normal weight	28.8	21.2	10.6	21.4	0.02
	25–30	28.8	21.9	28.5	32.7	
	≥30	42.3	57.0	61.0	45.9	
Abdominal obesity		71.4	89.0	94.1	84.4	0.001
Diabetes duration	Diabetes new case	29.1	11.0	2.4	20.2	<0.001
	<1 year	10.9	2.6	1.6	4.0	
	1–5 years	18.2	8.4	4.8	13.1	
	5–10 years	16.4	24.7	18.4	14.1	
	10–20 years	25.5	44.8	49.6	41.4	
	≥20 years	0.0	8.4	23.2	7.1	
Antidiabetic therapy	Non-insulin therapy	63.6	78.6	69.6	64.6	Ns
	Insulin therapy	50.9	57.8	64.8	40.4	0.01
HbA _{1c} ≥7%		87.3	92.9	89.5	81.6	Ns
Insulin secretion	Normal C-peptide	63.6	72.0	58.3	73.9	Ns
	Low C-peptide	12.7	14.0	11.1	4.3	
	High C-peptide	23.6	14.0	30.6	21.7	
Diabetic macrovascular complications	Overall	43.6	77.3	84.0	76.8	<0.001
	Peripheral artery disease	43.6	71.4	79.2	55.6	<0.001
	Ischemic heart disease	38.2	59.1	66.4	68.7	0.001
	Stroke	5.5	8.4	12.0	13.1	Ns
Diabetic peripheral neuropathy		69.1	84.8	91.5	85.9	0.002
Hypertension		80.0	83.1	91.2	89.9	Ns
Hypercholesterolemia		38.9	30.1	32.0	32.7	Ns
Hyper-LDL cholesterolemia		79.6	73.9	76.8	74.1	Ns
Hypertriglyceridemia		50.0	45.8	58.4	54.1	Ns
Hypo-HDL cholesterolemia		32.7	54.8	55.4	65.9	0.003
Hyperuricemia		9.6	15.7	31.2	25.3	0.002

was found only in 11.50% [14]. The high prevalence of DR and CKD identified in our study could be justified by the fact that hospitalized patients were more likely to have long lasting diabetes and multiple associated comorbidities.

The current study indicates that 11.0% of patients with isolated DR were patients with newly diagnosed DM and 10.3% of patients with isolated CKD were newly diagnosed DM. Similar to our findings, Hao Z. et al, indicated a high

prevalence of DR (20.3%) in patients with newly diagnosed T2DM [23]. The insidious onset of hyperglycemia symptoms that lead to late diagnosis of diabetes, after a long period of untreated hyperglycemia, but also other cardiometabolic risk factors (e.g. obesity, hyperuricemia) and lifestyle elements (e.g. smoking) are potential explanations for the increased prevalence of DR and CKD in newly diagnosed patients with diabetes [23, 24].

Table 5: Sociodemographic and biological parameters associated with diabetic retinopathy and/or chronic kidney disease.

Variables (Mean ± SD)	Without DR or CKD	DR alone	CKD associate with DR	CKD alone
Age	58.6 ± 12.2	60.3 ± 10.6	61.7 ± 9.9 [¥]	65.3 ± 11.3 ^{†, &}
BMI	28.8 ± 8.7	31.3 ± 8.2 [*]	31.7 ± 5.4 ^{#, ¥}	30.0 ± 7.9
Maximum BMI	32.2 ± 8.7	34.8 ± 8.4 [*]	35.2 ± 6.7 [#]	33.5 ± 5.6
Waist	100.1 ± 16.6	106.3 ± 15.5 [*]	109.2 ± 13.9 ^{#, ¥}	104.5 ± 16.3
DM duration	5.7 ± 5.9	11.1 ± 7.8 [*]	14.2 ± 7.2 ^{#, ‡, ¥}	9.2 ± 8.4 ^{†, &}
Insulin dose/kg	0.4 ± 0.3	0.5 ± 0.3	0.5 ± 0.4	0.5 ± 0.3
eGFR	90.2 ± 15.9	88.6 ± 13.9	64.4 ± 25.3 ^{#, ‡}	64.5 ± 22.6 ^{†, &}
SBP	137.1 ± 19.7	141.0 ± 19.7	142.6 ± 22.1	141.4 ± 19.4
DBP	83.3 ± 11.8	81.2 ± 12.6	79.6 ± 12.6	81.1 ± 11.2
Cholesterol	187.3 ± 58.1	179.0 ± 47.7	182.9 ± 48.4	189.5 ± 79.7
HDL cholesterol	38.7 ± 13.4	40.5 ± 12.8	37.6 ± 13.0 [‡]	38.2 ± 13.4
LDL cholesterol	105.8 ± 48.3	104.0 ± 44.2	99.4 ± 40.5	101.9 ± 39.8
Triglycerides	197.4 ± 150.2	165.6 ± 96.0	208.0 ± 128.2 [‡]	238.5 ± 427.1
Creatinine	0.8 ± 0.1	0.8 ± 0.1	1.3 ± 0.7 ^{#, ‡}	1.2 ± 0.9 ^{†, &}
Uric acid	4.4 ± 1.5	4.8 ± 1.4	5.8 ± 2.0 ^{#, ‡}	5.6 ± 1.8 ^{†, &}
HbA1c	10.1 ± 2.8	9.8 ± 2.0	9.6 ± 2.2	10.1 ± 2.7
C-peptide	2.9 ± 2.2	2.3 ± 1.4	3.3 ± 2.3	2.8 ± 1.5

*p < 0.05 DR alone vs. without DR or CKD; †p < 0.05 CKD alone vs. without DR or CKD; #p < 0.05 CKD associate with DR vs. without DR or CKD; &p < 0.05 RD alone vs. CKD alone; ‡p < 0.05 RD alone vs. CKD associate with DR; ¥CKD alone vs. CKD associate with DR.

Current study indicated that the simultaneous presence of DR and CKD were significantly associated with obesity and with abdominal obesity, which is consistent with other research results. Grey et al. reported that the increased risk of retinopathy correlates with higher BMI in a sample of 14,657 diabetic patients followed up for an average of 6.68 years [23, 25]. Data regarding the association of DR with obesity are conflicting, with studies reporting a lack of correlation and even an negative correlation between BMI and DR [26, 27]. Chan J. C. Y. et al. indicated that in patients with T2DM, a higher BMI appeared to have a protective effect on DR [27]. Man R. E. et al. shows that isolated abdominal obesity is a more clinically relevant risk factor for DR than BMI in T2DM patients [28]. Association of DR with obesity and abdominal obesity indicated the potential role of insulin resistance in the occurrence and progression of retinopathy.

Association of DR with CKD may be the key to understanding the pathogenic mechanisms common to microvascular complications of diabetes.

Future studies are needed to focus on establishing pathogenic mechanisms common to all microvascular complications as well as those specific to each microvascular complication so that physicians can effectively identify people at increased risk for developing and progression of chronic microvascular diabetic complications.

Lifestyle optimization, weight loss, obtaining and maintaining glycemic control, treating dyslipidemia and hyperuricemia should be important components of prevention programs and microvascular complications management.

The results obtained are of major importance for the medical system and can be of great help in the implementation of the microvascular

complications prevention programs, together with the reduction of the costs for treatment of these diseases.

Conclusions

The results of this study indicate a high prevalence of DR and CKD both isolated and associated in hospitalized T2DM patients, providing data on their prognosis and association with several cardiometabolic risk factors.

This finding suggests that it is essential to actively identify and combat the modifiable risk factors associated with microvascular diabetic complications in order to prevent or reduce their progression.

Conflicts of Interest

The authors declare no conflict of interest.

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Original Research

Pitfalls in establishing type of diabetes and optimal therapy: clinical cases series

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Received: 14 May 2021 / Accepted: 10 June 2021

Abstract

Type 1 diabetes is an autoimmune disease characterized by absolute endogenous insulin deficiency. We report three clinical cases which highlight the particularities of diagnosis and evolution of patients with type 1 diabetes. These three cases have been diagnosed in different circumstances. The first case with symptoms of hyperglycemia and inaugural diabetic ketoacidosis, without detecting a precipitating factor; the second case accidentally diagnosed, in the absence of hyperglycemia symptoms; and the last case in the context of symptoms of hyperglycemia and ketosis precipitated by a respiratory infection. The cases are characterized by the presence of autoimmunity markers (anti-GAD positive antibodies) and C-peptide within normal range. In dynamics, with the initiation of insulin therapy and the remission of glucotoxicity, the insulin requirement decreased progressively, in all patients therapeutic regimen being represented by a low dose of basal insulin, in the conditions of a C-peptide still within normal limits at 4 years (first case), 1 year (second case), respectively 2 years (third case) from the diagnosis and without significant glycemic variability registered on the continuous glycemic monitoring system. In the third case, there is a lack of regression of insulin requirements after remission of SARS COV-2 infection, and in the second case, the maintenance of prandial insulin secretion sufficient to induce hypoglycemia after administration of a minimal dose of prandial insulin, but insufficient to control post-prandial glycemia in the conditions of omitting the administration of prandial insulin. In each of the cases previously presented we highlight the existing pitfalls in establishing the diagnosis and especially the challenges in choosing the optimal therapy in patients with type 1 diabetes, as the evolution can sometimes be atypical.

Keywords: continuous glucose monitoring system, glycemic variability, insulin secretion, time in range, type 1 diabetes

Background and Aims

Type 1 diabetes mellitus (DM) is considered an autoimmune disease with multifactorial etiology, characterized by destruction of pancreatic beta cells, reaching in time the absolute endogenous insulin deficiency [1]. The rate of beta cell destruction is quite variable, being aggressive in some individuals (mainly infants and children) and slow in others (mainly adults) [2].

Autoimmune markers characteristic to type 1 DM includes: glutamic acid decarboxylase

(GAD) antibodies, tyrosine phosphatase antibodies (IA2A), insulin autoantibodies (IAA), islet cell antibodies (ICA) and the zinc transporter antibodies (anti-ZnT8 antibody) [1, 3].

Patients with type 1 diabetes are also at risk to develop other autoimmune disorders such as Hashimoto thyroiditis, Graves disease, celiac disease, Addison disease, vitiligo, autoimmune hepatitis, myasthenia gravis or pernicious anemia.

At first glance, type 1 diabetes pathophysiology and management might seem



straightforward, however, the more that is learnt about the disease, the less it seems is truly known. What once seemed like a single autoimmune disorder, originating in T cell-mediated β -cell destruction, is now recognized to result from a complex interplay between environmental factors and microbiome, genome, metabolism and immune system that vary between individual cases [4].

The current cases series presentation highlights the pitfalls in establishing the diagnosis, as well as the challenges of choosing the optimal therapy for patients with diabetes. Thus, a series of three clinical cases are presented in which the particularities of diagnosis and evolution of patients with diabetes are highlighted.

Materials and Methods

Case report no 1

On the first case, we present a 19-year-old patient, without a hereditary history of diabetes, obesity or cardiovascular diseases, diagnosed with type 1 diabetes mellitus 4 years ago, in the context of the signs and symptoms of hyperglycemia (polyuria, polydipsia and weight loss of approximately 12 kg within 1 year) and inaugural diabetic ketoacidosis without detecting a precipitating factor.

Physical exam at diagnosis revealed underweight (height 170 cm, weight 52 kg, body mass index [BMI] 17.99 kg/m²) and waist 68 cm.

Laboratory data at diagnosis revealed fasting plasma glucose 362 mg/dL, HbA_{1c} 13.01%, urinary ketone bodies 150 mg/dL (maximum laboratory detection), anti-GAD antibodies 135 IE/mL (normal value: 1.1–4.4 ng/mL). Screening for autoimmune thyroiditis was performed by dosing anti-thyroglobulin and anti-thyroperoxidase antibodies, the screening being negative. Fundoscopy did not reveal lesions of diabetic retinopathy. The rest of the explorations that could have highlighted the presence of a precipitating factor of ketoacidosis (EKG, kidney and liver function, urine culture, chest X-ray, ultrasound of abdomen, pelvis and thyroid) were within normal limits.

At diagnosis, basal bolus insulin therapy with Novo-Rapid (NR) insulin and Lantus insulin was initiated, with dose titration according to glycemic profile. The recommended insulin therapy regimen at hospital discharge was 6 IU NR-6 IU NR-5 IU NR-9 IU Lantus, the total daily insulin dose being 0.5 IU/kg. It was recommended glycemic self-monitoring and titration of insulin doses based on glycemic profile.

The patient was re-evaluated clinically and metabolically within 8 months of diagnosis, when a weight gain of 9 kg was detected, possibly secondary to frequent episodes of hypoglycemia supported by a significant reduction in HbA_{1c} (5.1%) and by reporting episodes of imperious hunger, remitted after ingestion of carbohydrates, the patient omitting the determination of blood glucose before the carbohydrates intake.

The potential explanation for hypoglycemic episodes is the lack of regressive titration of insulin doses in the context of the onset of transient remission of diabetes associated with decreased glucotoxicity.

Continuous glycemic monitoring was initiated and based on the evolution of the glycemic profile, regressive titration of insulin doses was performed, establishing the following therapeutic regimen: 4 IU NR-4 IU NR-4 IU NR-4 IU Lantus, the total daily insulin dose being 0.25 IU/kg.

On this therapeutic regimen, on the records of the continuous glycemic monitoring system, no important glycemic variables were highlighted, the percentage of time with glycemic values in target (70–140 mg/dL) being 97%, the coefficient of variation (CV) was 16.6%, no glycemic values lower than 70 mg/dL were registered, the percentage of time spent with glycemic values above the target (140 mg/dL) was only 3%, the maximum glycemic value being 162 mg/dL, without significant post-prandial glycemic excursions and without inter-day glycemic variability (Figure 1).

This insulin requirement was maintained relatively constant for a period of about 2 years, with no changes in body weight, and the HbA_{1c} value ranged between 5.1% and 5.8%.

The evolution of glycemic profiles imposed the gradual decrease of insulin doses, therefore at 4 years after diagnosis, the

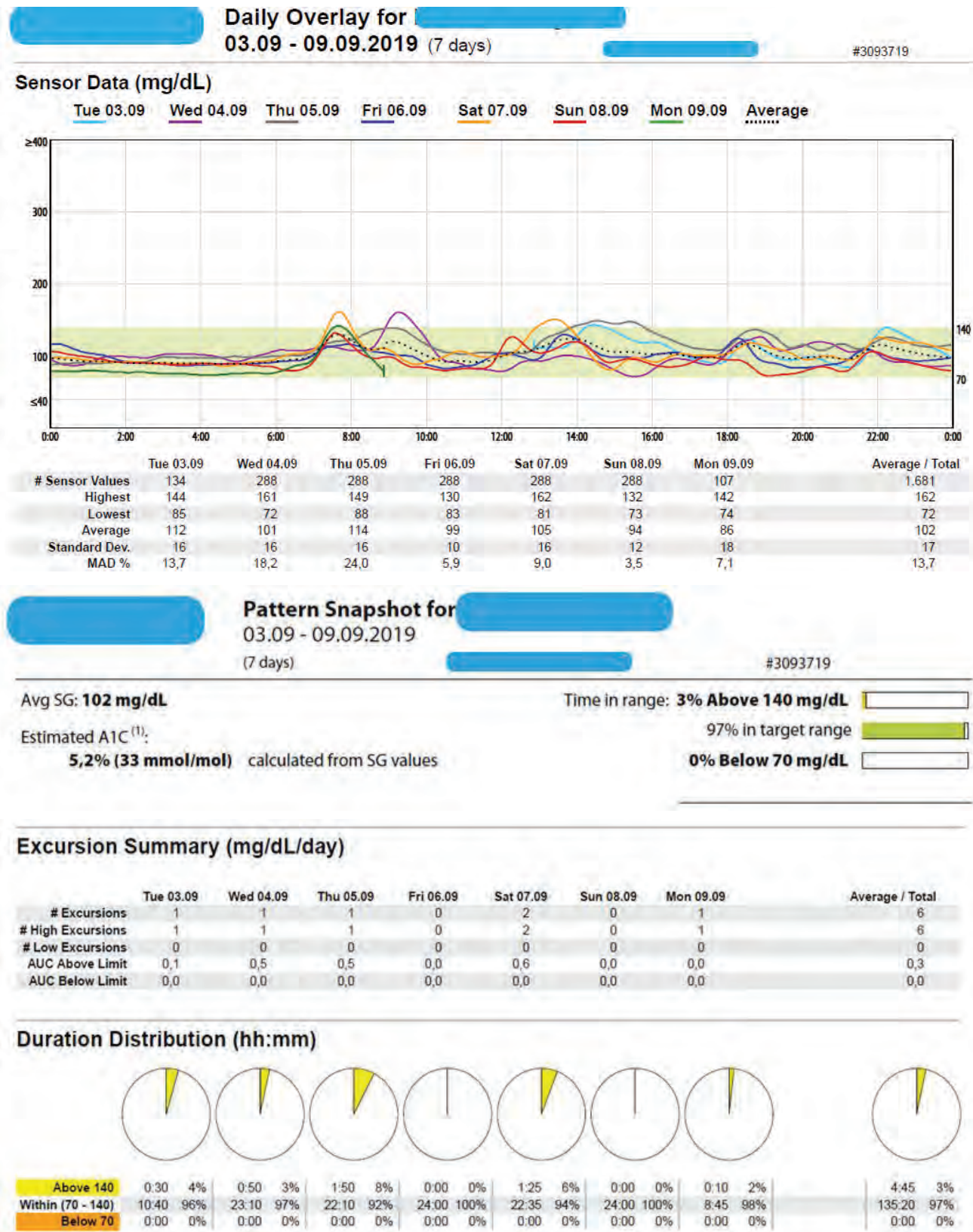


Figure 1: Glycemic profile, pattern snapshot and excursion summary assessed by continuous glucose monitoring system.

administration of prandial insulin was stopped, the patient's therapeutic regimen being represented only by basal insulin (3UI Lantus) administered in the morning.

Biological assessment after 6 months period with basal insulin therapy alone showed a normal C-peptide value – 1.9 ng/mL (normal value: 1.1–4.4 ng/mL), HbA_{1c} – 6%, and glyce-mic values in the established interprandial and post-prandial glycemic targets.

Case report no 2

In the second case, we present a 31-year-old patient, without a hereditary history of diabetes, obesity or cardiovascular disease, diagnosed a year ago with type 1 diabetes, by coincidence, in the absence of signs and symptoms of hyperglycemia, during routine investigations that showed fasting blood glucose 130 mg/dL and 6.4% HbA_{1c}.

The clinical evaluation revealed a normal weight (height 173 cm, weight 61 kg, BMI 20.3 kg/m², maximum BMI 23.7 kg/m²) and waist 82 cm.

Although at that time the classical criteria for diabetes diagnosis were not met, considering the young age of the patient, biological investigations were continued with the dosing of anti-GAD antibodies – >256 IE/mL (normal value < 10 IE/mL), C-peptide – 0.59 ng/mL (normal value: 0.8–3.8 ng/mL) and urinary ketone bodies are absent.

The fundus examination of eye was performed and did not show diabetic retinopathy. Basal-bolus insulin therapy was initiated with Fiasp and Tresiba insulin in doses that have been titrated according to the evolution of the glyce-mic profile up to a requirement of 3 IU Fi- 3 IU Fi- 3 IU Fi- 6 IU Tresiba (0.24 IU/kg).

After 1 year diagnosis, the patient presents for significant glyce-mic variability with frequent episodes of symptomatic severe hypoglycemia (glycemia 14 mg/dL), occurring post-prandial, even on the minimum dose of prandial insulin (1UI Fiasp administered preprandial for a quantity of 60–70 g carbohydrates), but with glyce-mic values that exceed the post-prandial targets in the absence of prandial insulin administration.

Also, glyce-mic self-monitoring indicated hyperglycemia at one hour post-prandial (440

mg/dL) justified by the omission of preprandial Fiasp administration and a high intake of concentrated sweets and fast food, hyperglycemia which suggested the need to administer 4 IU of Fiasp, administration that caused a sudden and significant drop in blood glucose level, reaching the value of 50 mg/dL within 45 minutes, accompanied by specific symptoms of hypoglycemia and difficult to correct by administration of sugar and carbohydrates.

Evaluation of insulin secretion indicated a low value of fasting C-peptide – 0.9 ng/mL (normal value: 1.1–4.4 ng/mL) and a normal value of C-peptide at 1 hour after a mixed lunch – 1, 39 ng/mL (normal value: 1.1–4.4 ng/mL).

Given both the evolution of the glyce-mic profile and the value of post-prandial C-peptide, it was recommended to stop the administration of prandial insulin, the administration of basal insulin (6 IU Tresiba) in the morning and the combination of alpha-glucosidase inhibitors (Glucobay 50 mg) before main meals. The favorable evolution of the glyce-mic profile was observed without detecting episodes of hypoglycemia and without important post-prandial glyce-mic excursions.

Case report no 3

In the last case, we present a 17-year-old patient with a hereditary history of type 2 diabetes on the paternal line (grandmother, great-grandmother), who was diagnosed 2.5 years ago with type 1 diabetes in the context of the signs and symptoms of hyperglycemia (polyuria, polydipsia and weight loss of 8 kg) with 2 months duration and ketosis which had as a precipitating factor an upper respiratory tract infection.

Clinical examination at diagnosis revealed normal weight (height 181 cm, weight 72 kg, BMI 21.98 kg/m²) and a normal waist (82 cm).

Para-clinical examinations at diagnosis revealed venous blood glucose 267 mg/dL, HbA_{1c} 10.5%, urinary ketone bodies 150 mg/dL (maximum laboratory detection), C-peptide 2.73 ng/mL (normal value: 1.1–4.4 ng/mL), C-peptide value should be interpreted in the context of glucotoxicity, anti-GAD antibodies 56 IE/mL

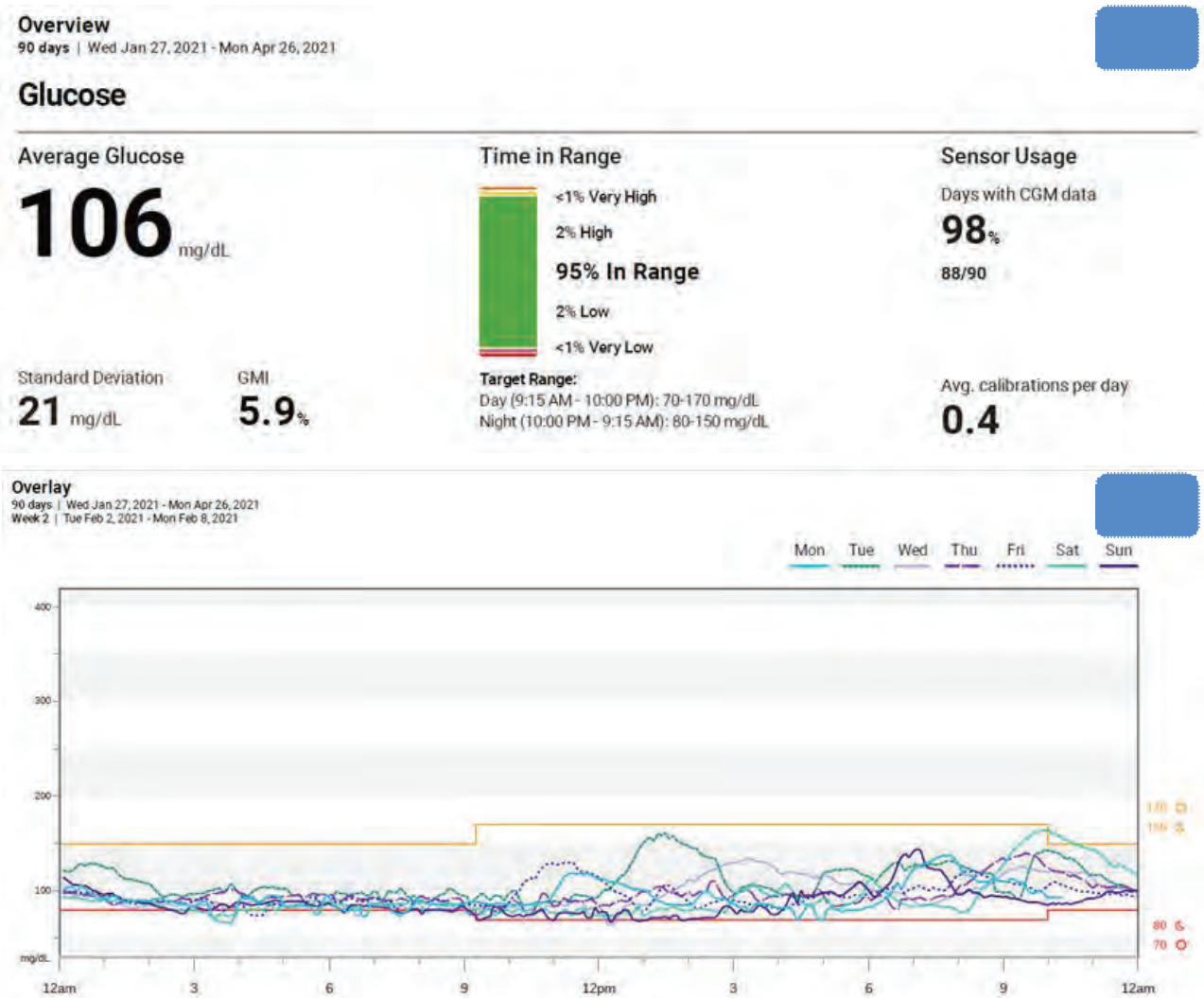


Figure 2: Glycemic profile and time in range overview (90 days period).

(normal value: <10 IE/mL) and anti-IA2 antibodies >4000 IE/mL (normal value: <10 IE/mL), the rest of the biological explorations being within normal limits.

Screening was performed for autoimmune thyroiditis by dosing anti-thyroglobulin and anti-thyroperoxidase antibodies and for celiac disease by measuring IgA, anti-gliadin and anti-endomysium tissue anti-transglutaminase antibodies, all antibodies being negative. Also, the diabetic retinopathy was excluded by performing the fundus examination.

At diagnosis, basal bolus insulin therapy was initiated with Novo-Rapid and Lantus insulin, in doses that were titrated based on the evolution of the glycemic profile up to a requirement of 12 IU NR-10 IU NR-8 IU NR-18 IU Lantus, the total daily insulin dose being 0.66 IU/kg.

In evolution, with the remission of glucotoxicity, insulin requirements decreased significantly, the administration of prandial insulin was stopped, the patient administering only basal insulin (4 IU Lantus) in the morning, C-peptide value remaining within normal limits (3.06 ng/mL at 5 months after diagnosis, respectively 1.34 ng/mL at 1 year after diagnosis, dosed at an HbA_{1c} value of 6.08% and 5.2%, respectively) (Figure 2).

This insulin requirement has remained relatively constant over approximately 2 years of evolution, increasing significantly during SARS COV-2 infection (January 2021) and remaining high after the remission of acute infectious phenomena, the therapeutic regimen being currently represented by basal insulin in a dose of 14 IU Lantus, to which is associated prandial insulin (1 IU for 10 g carbohydrates), under the

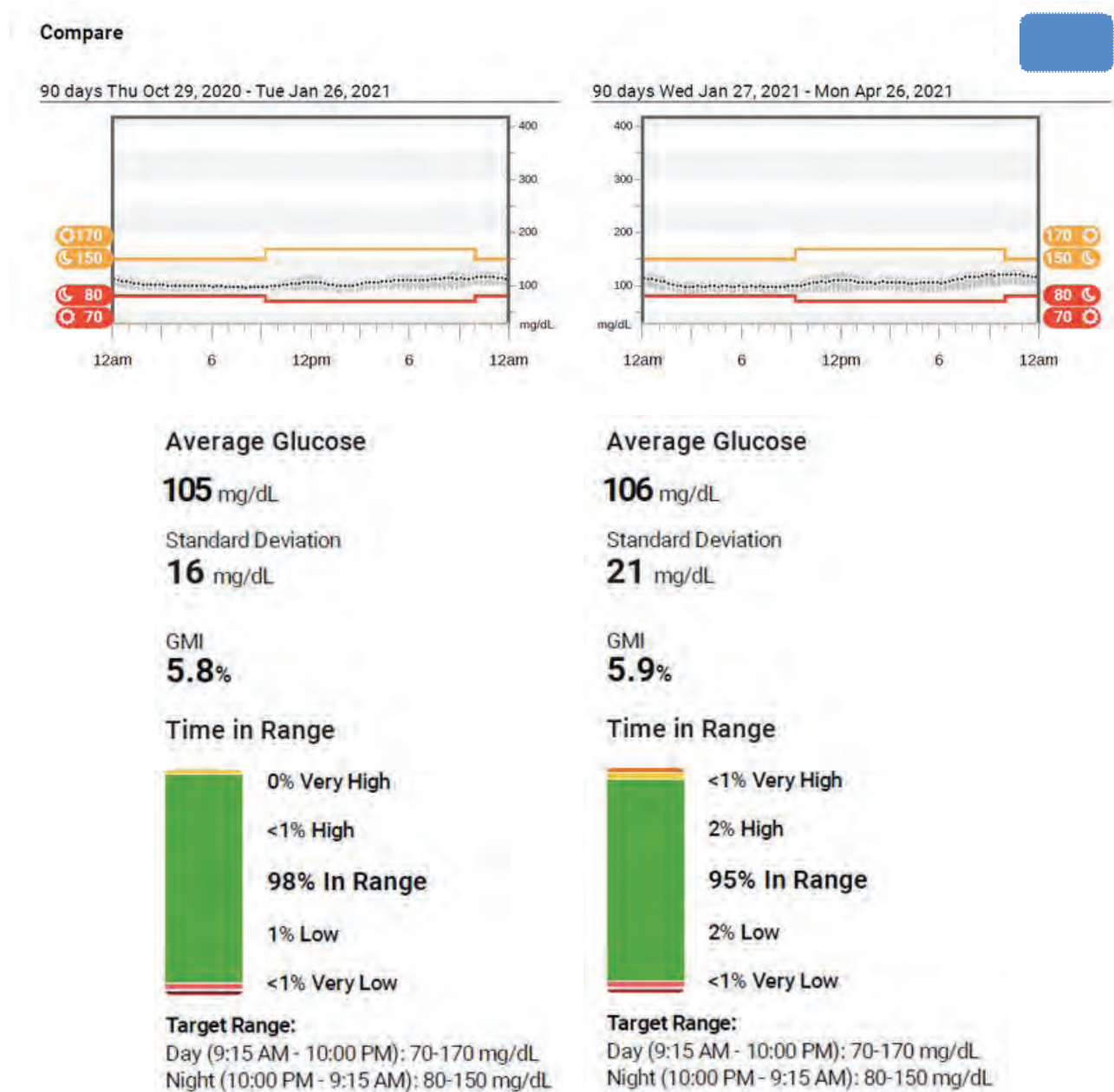


Figure 3: Glycemic variability and time in range before (left side) and after (right side) SARS COV-2 infection (January 2021).

conditions of a C-peptide of 1.27 ng/mL (normal value: 0.8–3.8 ng/mL) and a HbA_{1c} of 5.2%, without highlighting the important glycemic variability on the records of the continuous blood glucose monitoring system (Figure 3).

Discussions

Peculiarities of diagnosis and treatment of the presented cases

The peculiarity of the first case is represented by the slow and insignificant decline

of insulin secretion indicated by the value of C-peptide and the very low insulin requirement with maintaining an optimal glycemic control at 4 years from diagnosis, in the context of the patient's young age, identifying markers of autoimmunity. And the clinical features of the diabetes onset that would have suggested the rapid and permanent onset of insulinopenia by autoimmune destruction of pancreatic beta cells.

The peculiarity of the second case is the maintenance of prandial insulin secretion level at the lower limit of the normal range, which explains the occurrence of hypoglycemia after

administration of a minimum dose of prandial insulin and hyperglycemia in conditions of prandial insulin omission, associated with a low level of basal insulin secretion. Administration of alpha-glucosidase inhibitors allowed the correction of post-prandial blood glucose by reducing the intestinal absorption of monosaccharides without inducing hypoglycemia. Given that the current and maximum BMI was not higher than 25 kg/m², we did not opt for other therapeutic options targeting post-prandial blood glucose (inhibitors of sodium glucose transporter 2 – SGLT2).

The peculiarity of the third case is represented by the slow increase of insulin requirements in a young patient with a cluster of markers of autoimmunity, an evolution that can be explained by maintaining an efficient level of insulin secretion indicated by C-peptide values, clinical and biological characteristics presented at the time of diabetes diagnosis (ketosis precipitated by an infectious factor) but also the presence of hereditary antecedents of type 2 diabetes. The lack of regression of insulin requirements after remission of SARS COV-2 infection is also particular.

The evolution of insulin secretion and the maintenance of glycemic control in conditions of a low insulin requirement, for a relatively long period of time, in normal weight patients with markers of autoimmunity (anti-GAD antibodies) present, make it difficult to frame the type of diabetes not only in the traditional classification of diabetes, but also in the five clusters defined by Ahlqvist E. et al. [5].

The cases presented by us have characteristics such as diabetes with early onset, normal weight and the presence of anti-GAD antibodies, common to the first cluster reported by Ahlqvist E. et al., but, unlike patients in cluster 1, all three cases do not show marked insulin deficiency and have profiles glycemic in therapeutic targets on low doses of insulin after a long period from the diabetes onset.

The transitory clinical remission of type 1 diabetes usually occurs early after the onset of diabetes and typically lasts between 3 and 12 months [6, 7]. A potential explanation for transient remission is a reduction in glucotoxicity,

by initiating insulin therapy, which induces an increase in endogenous insulin secretion from surviving beta cells [8, 9].

Several recent studies indicated residual beta cell function after more than 5 years following the onset of type 1 diabetes [10]. Prolonged maintenance of residual insulin secretion is associated with low risk of severe hypoglycemia, diabetic chronic microvascular complications and achievement of long term glycemic control [11–15].

The molecular mechanism underlying remission in type 1 diabetes is not fully elucidated. Certain factors including unfavorable cytokine profile, immune mediators, genetic markers, increased glucagon secretion, acidosis severity and younger age at diagnosis and cluster of diabetes-associated autoantibodies represent predictors of the occurrence and duration of clinical remission [16–19].

The clinical and biological characteristics of the presented patients are polymorphic, including both predictors of non-remission but, at the same time, of a long period of remission.

Conclusion

The presented cases highlight the existing pitfalls in establishing the diagnosis, as well as the challenges of choosing the optimal therapy in patients with type 1 diabetes, as the evolution can sometimes be atypical.

Conflicts of interest

The authors declare no conflict of interest.

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Original Research

Prevalence of diabetes and diabetes complications in a south-eastern county from Romania

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Received: 11 May 2021 / Accepted: 10 June 2021

Abstract

Background and Aims: The literature confirms that, due to the global increase in the incidence of obesity and sedentary life-style, there has been an increase in the number of cases of type 2 diabetes (T2DM) among children. Thus, this pathology has the potential to become a global health problem with devastating effects on health. Through the present clinical research we sought to determine the role that individual variables play in increasing the risk of complications. **Materials and Methods:** This retrospective, consecutive case study, enrolled 2493 adult patients diagnosed with diabetes, attending scheduled visits in the diabetes outpatient or hospitalized in the Diabetes Clinic between January 2017 and December 2019. Data was collected from the patient's record and further analyzed using SPSS IBM Statistics V26 statistical package. **Results:** The peak of incidence of diabetes complications was in the 60–65 years age-group, respectively polyneuropathy (63.6% for newly diagnosed patients vs. 83.4% in patients with previously known diabetes). The most common comorbidities were arterial hypertension (61.7% for newly diagnosed patients vs. 64.3% in patients with previously known diabetes) and dyslipidemia (33.2% for newly diagnosed patients vs. 43.8% in patients with previously known diabetes). Complications were associated with poor glycemic control (serum blood glucose above 220 mg/dL, respectively HbA_{1c} of more than $9.36 \pm 2.2\%$). **Conclusions:** In our study, there was an increased number of complications, the most frequent being the polyneuropathy. Also, even the newly diagnosed diabetes patients had multiple comorbidities associated.

Keywords: adult patients, changes in eye examination, diabetes mellitus, diabetic neuropathy, microvascular complications

Introduction

Diabetes mellitus (DM) is a major public health problem that is rapidly approaching epidemic proportions worldwide. The International Diabetes Federation estimates an increase in the

number of cases to 578 million by 2030, compared to 463 million 2019 [1, 2]. In this study, a major concern is the increasing incidence of type 2 diabetes (T2DM) in young people, including the presence of obesity among pre-adolescents. In the case of developed countries, most diabetics



are older than 64 years, compared to developing countries, where the most affected people are in the productive years of their lives, aged between 35 and 64 years [3].

Approximately 7 million new cases of diabetes are reported annually, and the most dramatic increases in T2DM have occurred in populations that have experienced sudden and major lifestyle changes [4]. Numerous researches in the field have concluded that diabetes remains one of the main pathologies incriminated for deaths worldwide, this being largely due to the presence of subsequent complications.

At the same time, studies show that one of the most common complications of diabetes, as seen in the observational analysis of patient progression is represented by diabetic neuropathy, while diabetic retinopathy is the leading cause of visual impairment among diabetic patients [1, 5]. The existence of the so-called diabetic foot is due to pathophysiological changes in the circulatory system and also to nerve damage. The corroboration of all these elements proves that the risk of ulcers and even amputation of the limb (total or segmental) is an element to be taken into account, a continuous concern of specialists in the field. Research shows that diabetes is the most common cause of non-traumatic lower limb amputation [1, 5].

According to studies, the evolution of patients diagnosed with diabetes includes the risk of these complications in a period of about 15 years from the time of diagnosis. Thus, after this time, even if the subjects declare the existence of optimal glycemic control, it can be seen that 2% of patients completely lose their visual capacity, while about 10% develop a severe visual impairment [1, 5].

Diabetes is also recognized as the main promoter of renal failure, although it is important to remember that its incidence is dependent on two factors: the severity of symptoms and the duration of the disease [6, 7].

According to clinical studies in the literature, in both patients with type I diabetes and those with T2DM, it is highlighted that HbA_{1c} may be responsible for increasing the incidence of diabetes complications, being also considered a marker for the development of microangiopathic

pathologies and, at the same time, a risk factor for cardiovascular diseases. Glycolized hemoglobin has a very high specificity, compared to FPG (fasting plasma glucose) and OGTT (oral glucose tolerance test), its sensitivity is lower [8].

According to data from 2019, it is estimated that by 2030 the number of patients diagnosed with diabetes will reach 578.4 million, and by 2045 about 700.2 million adults aged 20–79 years will be diagnosed with diabetes. It is also estimated that 351.7 million people in the 20–64 years age group suffer from diabetes, whether diagnosed or undiagnosed [9].

The present study aimed to evaluate the impact of a several parameters in the risk of mortality among patients with known type 1 or type 2 diabetes. Socio-demographic variables were analyzed to determine the role of socio-economic implications, as well as the prevalence of diabetes. This will emphasize the need for a plan to prevent it, or at least reduce the risk of unfavorable developments for patients in the study group. At the same time, aimed to analyze in a comparative way the incidence of complications, in relation to the duration of diabetes.

Material and Methods

Study design This clinical research received the approval of the Ethics Commission of the hospital unit. Data of interest was collected, during a 3-years timeframe, from January 2017 to December 2019. In the study 2493 adult patients, of which 371 were newly diagnosed, were enrolled. Each of these patients completed a study participation agreement, prepared according to the Helsinki Declaration.

As inclusion criteria – the age of patients over 18 years, patients with a definite diagnosis of diabetes, subjects who accepted participation in this study, by signing an informed consent.

Statistical analysis

The present study possesses the characteristics of an observational, retrospective research, performance based on the information

obtained by analyzing the observation sheets of patients diagnosed with diabetes. The data processing was performed in the statistical program SPSS IBM Statistics (V.26) performing contingency and frequency tables, after centralizing the information using Microsoft Excel 2020.

Results and Discussions

The final group was formed as previously stated from a number of 2493 patients, divided in two subgroups; subgroup A: 371 patients with newly diagnosed diabetes (14.9%) and subgroup B: 2122 patients previously diagnosed with diabetes (85.1%) already in the databases of Brăila county.

The maximum incidence of presentations, as a whole was detected in 2019, however, discussing separately for each subgroup in question, it will be noted that the two incident curves have different slopes during the study. Subgroup A is characterized by an upward curve, with approx. tripling of cases by the end of 2019 (with a percentage difference of 28.84% compared to the number of cases in 2017), while subgroup B has a decreasing curve (32.57% of patients known to DM). Sig.=0.000 * confirms that there are statistically significant differences between the years of presentation and the two subgroups. From the point of view of the distribution by sex, the predominance of female subjects is noticeable (55.3% of the patients of subgroup A, respectively 61.3% of subgroup B). The chi-square test confirms by $MR < 0.29$ the existence of statistically significant differences. The urban environment predominates in the case of patients from subgroup B (53.2%) – a fact justifiable from the perspective that DM is a pathology that requires regular checks and the continuous administration of treatment (sig. 0.172).

The mean age of patients in subgroup A is 61.82 years, with $SD \pm 13.37$ years, with a maximum age of 89 years. In subgroup B patients have ages between 18 and 97 years (63 ± 12.67 years). Differences in the number of hospitalization days were observed as follows: subgroup A has a maximum of 17 days of hospitalization (mean $6.95 \text{ days} \pm 2.87 \text{ years}$), while subgroup B subjects required stabilization. A maximum number of 65

days of hospitalization ($6.79 \text{ days and } SD \pm 3.16 \text{ days}$) (Table 1).

In both subgroups overweight patients predominate, followed by those with grade I obesity. The HbA_{1c} value does not show significant differences between the two population groups: $10.85 \pm 2.45\%$ for newly diagnosed patients, respectively, $9.15 \pm 2.18\%$ for subgroup B. Blood glucose was $264.57 \pm 70.18 \text{ mg/dL}$ for subgroup A, while for subgroup B blood glucose was $216.03 \pm 62.56 \text{ mg/dL}$. None of the above variables represent statistically significant differences compared to the two groups (Table 2).

In Table 3 the changes detected in the ophthalmological examination by eye examination are presented. There were statistically significant differences for a number of variables as follows: normal eye examination according to age (sig = 0.000 *), the presence of retinopathy (sig. 0.000 *), proliferative retinopathy (sig. 0.000 *), but also angiopathy (sig. 0.000 *).

Discussions

Complications of diabetes occur at the systemic level, and diabetes is still one of the leading causes of cardiovascular morbidity and mortality, kidney failure and amputation. In addition, studies have found an important association between early diagnosis of T2DM (15–40 years) and the more aggressive form of the disease, with premature development of complications [6].

Type 2 diabetes consists of a series of dysfunctions characterized by hyperglycemia, resulting from a combination of insulin resistance, inadequate insulin secretion, and excessive or inadequate glucagon secretion. Therefore, inadequate control of T2DM is associated with a number of microvascular, macrovascular and neuropathic complications [10].

The total lack of insulin hormone or resistance to this hormone means that glucose will remain free in the bloodstream. Over time, high blood glucose levels cause tissue damage, leading to the development of disabling and life-threatening complications [6, 11].

In recent years, more detailed analyzes of beta cell response and regulation have shown

Table 1: Diabetes groups depending on the time of diagnosis.

Diabetes groups depending on the time of diagnosis									
Newly diagnosed diabetes (subgroup A)					Patients known to have diabetes at the time of registration				
	Count	Mean	Row valid n%	Column valid N%	Count	Mean	Row valid n%	Column valid N%	Sig. (Chi parat)
The years in which the presentations took place	2017	70	8.5	18.9	757		91.5	35.7	0.000*
	2018	124	15.5	33.4	674		84.5	31.8	
	2019	177	20.4	47.7	691		79.6	32.6	
Gender of patients enrolled in the study	Female	205	13.6	55.3	1300		86.4	61.3	0.029*
	Male	166	16.8	44.7	822		83.2	38.7	
The origin of the patients enrolled in the study	Urban	183	14.0	49.3	1128		86.0	53.2	0.172729
	Rural	188	15.9	50.7	994		84.1	46.8	
Age of patients at the time of registration (expressed in years completed)			62			62			
	<20	2		0.5	10		83.30	0.5	0.005*
	20-30	7		1.9	37		84.10	1.7	
	30-40	12		3.2	90		88.20	4.2	
	40-50	65		17.5	238		78.50	11.2	
	50-60	59		15.9	499		89.50	23.5	
	60-70	131		35.3	714		84.50	33.6	
	70-80	74		19.9	431		85.30	20.3	
	>80	21		5.7	103		83.10	4.9	

Table 2: Diabetes groups depending on the time of diagnosis.

		Diabetes groups depending on the time of diagnosis							
		Newly diagnosed diabetes (subgroup A)				Patients known to have diabetes at the time of registration			
		Count	Mean	Row valid n%	Standard deviation	Count	Column valid N%	Mean	Standard deviation
IMC Groups	Underweight	4		1.1		35	1.7		
	Normal weight	70		18.9		425	20.2		
	Overweight	135		36.5		761	36.2		
	Grade I obesity	103		27.8		576	27.4		
	Grade II obesity	34		9.2		204	9.7		
	Morbid obesity	24		6.5		102	4.9		
HbA1C			10.85		2.45			9.15	2.18
Blood glucose values			264.57		70.18			216.03	62.56

Table 3: Diabetes groups depending on the time of diagnosis.

		Diabetes groups depending on the time of diagnosis					
		Newly diagnosed diabetes		Patients known to have diabetes at the time of registration		Sig.	
		Count	Column valid n%	Count	Column valid N%		
Associated with hypertension	No	142	38.3	757	35.70	0.336	
	Yes	229	61.7	1365	64.30		
Associated with polyneuropathy	No	135	36.4	353	16.6	0.000*	
	Yes	236	63.6	1769	83.4		
Dyslepidemic changes	No	123	33.2	929	43.8	0.000*	
	Yes	248	66.8	1193	56.2		
Occurrence of chronic kidney disease	No	371	100	2115	99.7	0.268	
	Yes	0	0	7	0.3		

*=statistically significant

that most subjects at risk for T2DM, i.e. those with resting glucose disorders and glucose tolerance, already have a significant loss of about 80% of the ability of the pancreas to secrete insulin [7].

According to the literature, the application of medical interventions for the prevention of T2DM may be the only way to prevent its complications. The most common chronic

complications of diabetes are represented by: cardiovascular spectrum pathologies, stroke-type neurological disorders and even cognitive disorders, chronic kidney disease, diabetic neuropathy, diabetic retinopathy, diabetic foot [4, 5].

Due to its chronic nature, as well as the severity of complications and the means needed to control them, diabetes is an expensive

pathology, both for the patient and his family, and for the health system [12, 13]. Diabetic foot is due to pathophysiological changes in the circulatory system and nerves, which often leads to ulceration and even amputation of the limb. Research shows that diabetes is the most common cause of non-traumatic lower limb amputation [14, 15].

The study limitations are: lack of application of a questionnaire, hence lack of subjective analysis and insufficient documentation of the patient's evolution due to lack of information.

As future research perspectives, it is suggested that to continue the analysis of the incidence of symptoms for drawing a profile of the adult patient (elderly and young) diagnosed with diabetes and carrying out clinical studies, through detailed collaboration with other specialties, on the role and effectiveness of treatment in preventing complications associated with DM.

Conclusions

There is an increased prevalence of complications in the whole group (with the predominance of cases in subgroup B subjects), with a peak incidence around the age of 60–65 years. At the level of the analyzed lot the highest share of patients had hypertension (independent of the subgroup belonging 61.7% subgroup A, respectively 64.3% subgroup B) without statistically significant differences (sig. 0.336); polyneuropathy is mostly detected as a complication of patients with old diabetes (83.4%) (sig.0.000 *); alteration of lipid metabolism was detected predominantly in newly diagnosed patients (66.80%, compared to 56.2% subgroup B) (sig.0.000 *). Complications were associated with the evolution of DM (newly diagnosed, or with variable age) and with a poor glycemic control (serum blood glucose above 220 mg/dL and HbA_{1c} above 9.36 ± 2.2%). The predominance of cases in subgroup B in the urban environment suggests an increased attention on the evolution of patients, respectively a higher incidence of female patients.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

Combined effects of κ -carrageenan and monosodium glutamate food additives: effect on free radical oxidation

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Received: 30 November 2020 / Accepted: 12 January 2021

Abstract

Background and aims: The universal mechanism that plays a key role in the implementation of the action of most toxic agents is the activation of free radical processes and the development of oxidative stress. Therefore, the aim of our study was to evaluate the indicators of lipid peroxidation and antioxidant defense system in rat tissues using a solution of κ -carrageenan, monosodium glutamate (MSG), and their combined effects to establish possible relationships. **Materials and methods:** The study was performed on 48 white non-linear male rats that were divided into 4 groups: 1st – control (intact animals); 2nd – animals that were intragastrically injected with κ -carrageenan at a dose of 40 mg/kg for 1 month; 3rd – animals that were intragastrically injected with MSG at a dose of 50 mg/kg for 1 month; 4th – animals that were intragastrically injected with κ -carrageenan and MSG in the above doses. We tested the levels of diene conjugates (DC), active products of thiobarbituric acid (TBA), superoxide dismutase (SOD) and catalase activities (CAT). **Results:** The highest activation of free radical oxidation was observed in lung tissues with the use of MSG and the combined effect of κ -carrageenan and MSG, whereas the lowest studied indicators of lipid peroxidation were registered in all tissues of group 1. The lowest values of the antioxidant defense system parameters were found in 3-d group, while the use of κ -carrageenan caused an increase of SOD and CAT activity with the highest values in liver tissues. **Conclusions:** The combined use of a solution of κ -carrageenan and MSG has a negative effect on the processes of free radical oxidation, which is manifested by a statistically significant increase of lipid peroxidation products levels in blood serum, lungs and liver and decreased activity of antioxidant system enzymes in the lungs and liver and in the process significant medium-strength correlations between pro- and antioxidants were established.

Keywords: antioxidant defense system, combined use, κ -carrageenan, lipid peroxidation, monosodium glutamate

Background and Aims

Food additives are widely used in the food industry to maintain food quality, achieve homogeneity, enhance flavor or improve the texture of food [1]. The second place in terms of consumption of additives after the food industry is occupied by the cosmetic industry for the manufacture of lotions, creams, shampoos [2]. Food

additives are also used in the textile industry, in biotechnological production for cell immobilization and as a substitute for bacteriological agar [3]. With the expansion of the food additives production, the range of food and industrial products obtained without their use is constantly decreasing [4, 5].

In recent decades, carrageenans have become one of the most popular hydrocolloids



in the food industry [6]. It should be noted that currently the carrageenans have the highest commercial total production (about 60 thousand tons per year), which is 626 million US dollars in annual profit [7]. Their main purpose is the formation of gels, so carrageenans are widely used as emulsifiers, stabilizers, thickeners [8]. Given that carrageenans belong to soluble dietary fibers, they are involved in the regulation of homeostasis, have immunostimulatory and anticoagulant properties, which makes them interesting for use in functional foods [9].

Monosodium glutamate (MSG, monosodium salt of glutamic acid, E621) is used in most food technologies as a food additive and the amount of its use is not controlled. In Ukraine, only in 2000, after the resolution of the Cabinet of Ministers №342 of February 17, MSG was included in the list of permitted food additives in Ukraine [10]. At the same time, the safe daily dose of food additives is ambiguous. Nowadays, there are no reliable data that would show in what doses and under what conditions MSG, consumed constantly in the form of additive E621 is harmful to health [11, 12]. Literature data on the toxic effects of MSG are few and more related to the development of MSG-induced obesity [13–16] and neurotoxicity [17, 18].

Although the safety of carrageenan in food has been debated for decades, E407 retains the status of a safe additive “Generally Regarded as Safe”. The Joint FAO/WHO Expert Committee on Food Additives has set its permissible daily dose – up to 75 mg per 1 kg of body weight [19]. In 2015, the Joint Expert Committee on Food Additives of the WHO concluded on the safety of carrageenan in infant formulas [20]. However, there are conflicting data on the safety of carrageenan. A number of studies have shown that carrageenan activates inflammatory signaling pathways, which leads to the induction of pro-inflammatory cytokines [21, 22]. Other studies show that carrageenans pass through the intestinal epithelium; are transferred through a portal blood flow to a liver; bind to TLR4 and induce pro-inflammatory reactions, as well as suppress insulin sensitivity [23].

The universal mechanism that plays a key role in the implementation of the action of most

toxic agents is the activation of free radical processes and the development of oxidative stress [24]. However, data on free radical oxidation in body tissues with the consumption of MSG, κ -carrageenan and their combined effects are extremely scarce, so there is a need to study this problem in detail.

Therefore, the aim of our study was to evaluate the indicators of lipid peroxidation and antioxidant defense system in rat tissues using a solution of κ -carrageenan, MSG, and their combined effects to establish possible relationships.

Materials and Methods

The study was performed on 48 white non-linear male rats that were kept on the standard vivarium diet of I.Horbachevsky Ternopil National Medical University. During the work the principles of the European Convention for the Protection of Laboratory Animals were followed. Experimental rats were divided into 4 groups: group 1 – control (intact animals); group 2 – animals that were intragastrically injected with κ -carrageenan at a dose of 40 mg/kg, dissolved in 0.5 mL of distilled water at room temperature for 1 month [25, 26]; group 3 – animals that were intragastrically injected with MSG at a dose of 50 mg/kg, dissolved in 0.5 mL of distilled water at room temperature for 1 month [27]; group 4 – animals that were intragastrically injected with κ -carrageenan and MSG in the above doses.

To prepare 10% of the homogenate, the lung and liver samples, that were taken immediately after euthanasia, were cooled in physiological saline to 1–3°C, dried with filter paper, then scissored and homogenized in 0.05 M Tris-HCl buffer (pH 7.4) using a magnetic homogenizer SilentCrusher S, (Heidolph, Germany) in a ratio of 1:9 (tissue weight: buffer volume). The resulting homogenate was centrifuged for 30 minutes at 3000 rpm in a centrifuge with cooling Hermle Z 32 HK. The supernatant was used for research [28].

For the study in erythrocyte hemolysates, whole blood was centrifuged at 3000 rpm for 15 minutes, the serum was collected, and erythrocytes were washed twice with isotonic sodium chloride solution. The method of Nishikimi N.

and co-authors was used to prepare hemolysate of erythrocytes [29]. According to the method, to 0.1 mL of washed erythrocytes were added 0.9 mL of Tris-HCl buffer 0.05 M (pH 7.4), 0.25 mL of ethyl alcohol, 0.15 mL of chloroform and the resulting mixture was stirred, centrifuged in a centrifuge for 15 minutes at 3000 rpm with cooling Hermle Z 32 HK. The supernatant was used for research.

The content of diene conjugates (DC) in blood serum (tissue homogenates) was evaluated by the classical method of Z. Placer (1968) in the modification of V.B. Havrylov, M.I. Mishkorudnaya (1983) [30]. To 0.2 mL of serum was added 2.0 mL of isopropanol-heptane mixture (in a ratio of 1: 1). The resulting mixture was shaken for 1 hour, then was added 0.5 mL of hydrochloric acid at pH 2.0, shaken for 2 minutes, then was added 1.0 mL of heptane and continued to shake for 15 minutes. After about 1 hour, photometry of the upper phase was performed at 232 nm against the control sample. The calculation was performed using the molar extinction coefficient of $2.20 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ and expressed in $\mu\text{mol/L}$ of blood serum or $\mu\text{mol/kg}$ of tissue.

The content of active products of thiobarbituric acid (TBA) in the serum was evaluated by the method of M. Uchiyama and M. Mihara [31]. To do this, 0.2 mL of serum was mixed with 2.0 mL of 1.4% solution of orthophosphoric acid and 1.0 mL of 0.5% solution of TBA acid. The mixture was incubated in a water bath for 45 minutes, then cooled and then 2.0 mL of n-butanol was added to extraction of the stained complex. The test tubes were shaken and centrifuged for 20 minutes at 4000 rpm. The upper phase photometry was performed at 532–570 nm against the control sample. The calculation was performed using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ and expressed in $\mu\text{mol/L}$ of blood serum or $\mu\text{mol/kg}$ of tissue.

To determine the superoxide dismutase (SOD) activity in hemolysates of erythrocytes was used the method of S. Chevari and co-authors [32]. To do this, to 0.2 mL of erythrocyte hemolysate supernatant (supernatant of lung or liver homogenate) were added 1.3 mL of pyrophosphate buffer (pH = 8.3, molar concentration 0.1 mol/L), 1.0 mL of nitro blue tetrazolium solution, 0.3 mL of phenazine methosulfate solution

and 2.0 mL of NADN_2 solution (molar concentration $0.2 \mu\text{mol/L}$). Next, the samples were kept in a dark place for 10 minutes and photometered at a wavelength of 540 nm on a SF-46 in a 1 cm cuvette against samples without NADN_2 . Samples with 0.2 mL of phosphate buffer were used as controls instead of hemolysate or homogenate. Enzyme activity was calculated by the formula $A_{\text{sod}} = T / (100\% - T) / C$ (where A_{sod} is SOD activity; T is the percentage of inhibition, C is the protein concentration), and the percentage of inhibition is $T = (E_k - E_d) \times 100 / E_k$ (where T is the percentage of inhibition; E_k is the extinction of the control sample; E_d is the extinction of the test sample). The protein content in body tissues and erythrocyte lysate was determined by the Lowry method [33]. The activity of SOD was expressed in conventional units per 1 mg of protein (con. units/mg of protein).

To determine the catalase activity (CAT) in the supernatant of erythrocyte hemolysate and lung and liver homogenate, a spectrophotometric method was used, which is based on the ability of the enzyme to highly efficiently catalyze the decomposition reaction of hydrogen peroxide to water and oxygen [34]. Phosphate buffer, hydrogen peroxide and Na_2EDTA were added to the control and test samples containing the test enzyme solution. The test tubes were left at room temperature for 10 minutes, and the reaction in the control sample was stopped by adding 1.0 mL of 10% sulfuric acid solution. An appropriate volume of sulfuric acid was added to the test sample after finishing of incubation. CAT was determined on a SF-46 spectrophotometer at a wavelength of 250 nm. The blank sample to account for the spontaneous decomposition reaction of hydrogen peroxide differs in that instead of a sample containing catalase, the same volume of phosphate buffer is added to it. The unit of activity was the amount of hydrogen peroxide that was decomposed during incubation per unit time. The CAT was expressed in μmol of hydrogen peroxide for 1 minute per 1 mg of protein.

Statistical processing of the results was performed using the computer program STATISTICA 7.0. The choice of the method of statistical research was based on the correct distribution of the studied features. Due to the

Table 1: Indicators of DCs in body tissues of rats ($\mu\text{mol/L [kg]}$) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	2.80 (2.58; 3.13)	4.15 (3.90; 4.23)	4.55 (4.25; 4.83)
Group 1	3.45^ (3.28; 3.60)	5.05^ (4.80; 5.23)	5.90^ (5.80; 6.10)
Group 2	4.6^ (4.48; 4.93)	6.15^ (5.88; 6.40)	6.65^ (6.38; 6.88)
Group 3	6.10^ (5.80; 6.50)	7.10^ (6.88; 7.23)	8.05^ (7.68; 8.33)
Kruskal-Wallis coefficient, p	H = 9.89; p = 0.02*	H = 8.52; p = 0.04*	H = 15.22; p = 0.002*

Notes: *Statistically significant results; ^The probability of differences relative to control.

incorrect distribution of quantitative characteristics, their descriptive statistics was carried out as reported as medians and interquartile range (IQ, percentile 25 and percentile 75).

Comparative analysis of quantitative indicators in three or more groups was performed using the Kruskal-Wallis test, which was considered statistically significant at its values of $p < 0.05$. Further pairwise comparison of groups was performed using the Mann-Whitney U test, taking into account the Bonferroni correction when assessing the level of statistical significance.

Results

It was found that the level of DC in the blood, lung and liver homogenates of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 1). In the serum of experimental animals, the level of DC of group 1 probably exceeded the control values by 23.21%, accordingly, group 2 – by 64.29% and group 3 – by 117.86%. In the lung homogenate, the level of DC of group 1 probably exceeded the control values by 21.69%, accordingly, group 2 – by 48.19% and group 3 – by 71.08%. In liver tissues, the level of DC of group 1 probably exceeded the control values by 29.67%, accordingly, group 2 – by 46.15% and group 3 – by 76.92% (Table 1).

The level of DC in the tissues of experimental animals apparently differed in the blood serum and lung tissues of rats of the experimental groups 1 and 2, 1 and 3 with the highest value by the combined use of κ -carrageenan and MSG (Table 2).

It was found that the level of active products of TBA in the blood, lung and liver homogenates of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 3). In the blood serum of experimental animals, the level of TBA of group 1 probably exceeded the control values by 33.75%, accordingly, group 2 – by 58.63% and group 3 – by 95.96%. In the lung homogenate, the level of DC of group 1 probably exceeded the control values by 30.23%, accordingly, group 2 – by 104.65% and group 3 – by 148.84%. In liver tissues, the level of DC of group 1 probably exceeded the control values by 19.10%, accordingly, group 2 – by 37.08% and group 3 – by 56.18% (Table 3).

Table 2: Levels of reliability (p) in the multiple comparison of DCs between the studied groups.

	Group 1	Group 2	Group 3
Serum			
Group 1	—	0.025*	0.01*
Group 2	0.025*	—	0.07
Group 3	0.01*	0.07	—
Lungs			
Group 1	—	0.03*	0.046*
Group 2	0.03*	—	0.27
Group 3	0.046*	0.27	—
Liver			
Group 1	—	0.37	0.03*
Group 2	0.37	—	0.03*
Group 3	0.03*	0.03*	—

Note: *Statistically significant results.

Table 3: Indicator of active products of TBA in body tissues of rats ($\mu\text{mol/L}$ [kg]) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	3.22 (3.08; 3.34)	2.15 (2.08; 2.43)	4.45 (4.10; 4.65)
Group 1	4.30 [^] (4.10; 4.50)	2.80 [^] (2.60; 3.10)	5.30 [^] (5.08; 5.60)
Group 2	5.10 [^] (4.98; 5.23)	4.40 [^] (4.18; 4.50)	6.10 [^] (5.95; 6.40)
Group 3	6.30 [^] (6.08; 6.60)	5.35 [^] (5.10; 5.88)	6.95 [^] (6.78; 7.13)
Kruskal-Wallis coefficient, p	H = 9.19; p = 0.03*	H = 11.62; p = 0.009*	H = 15.27; p = 0.002*

Notes: *Statistically significant results; [^]The probability of differences relative to control.

The level of TBA in the tissues of experimental animals apparently differed in the blood serum of rats of all experimental groups, in the lung homogenate in rats of groups 1 and 2, 1 and 3, in the liver tissue in rats of groups 1 and 3, 2 and 3 with the highest value by the combined use of κ -carrageenan and MSG (Table 4).

When comparing the indicators of LPO (relative to control), the highest values of the studied indicators were found in 3 experimental groups, where the highest activation of free radical oxidation was observed in lung tissues with the use of MSG and the combined effect of κ -carrageenan and MSG, whereas the lowest studied indicators of LPO were registered in all tissues of experimental group 1 (Figure 1).

It was found that the level of SOD activity in the homogenate of the lungs and liver of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 5). In the blood serum of experimental animals, the level of SOD activity in all experimental groups did not differ statistically significantly from the control. In lung tissues, SOD activity was significantly higher in animals

Table 4: Levels of reliability (p) in the multiple comparison of the active products of TBA between the studied groups.

	Group 1	Group 2	Group 3
Serum			
Group 1	—	0.045*	0.001*
Group 2	0.045*	—	0.03*
Group 3	0.001*	0.01*	—
Lungs			
Group 1	—	0.03*	0.046*
Group 2	0.03*	—	0.27
Group 3	0.046*	0.27	—
Liver			
Group 1	—	0.37	0.03*
Group 2	0.37	—	0.03*
Group 3	0.03*	0.03*	—

Note: *Statistically significant results.

of group 1 (by 21.77%) and, accordingly, lower in animals of group 2 (by 23.39%) and group 3 (by 56.18%) against control values. In liver tissues, the activity of SOD was significantly higher in animals of group 1 (by 40.74%) and, accordingly,

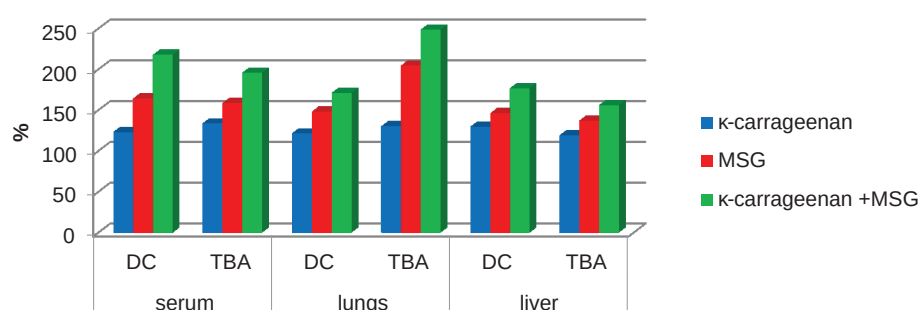


Figure 1: Comparison of lipid peroxidation indicators (%) in the tissues of rats under the combined effects of food additives.

lower in animals of group 2 (by 25.23%) and group 3 (by 59.95%) against control values (Table 5). The activity of SOD in the lungs of rats apparently differed in animals of groups 2 and 3 ($p = 0.01$) and, accordingly, in the liver of rats – in groups 2 and 3 ($p = 0.03$) with the highest values by the combined use of κ -carrageenan and MSG.

It was found that the level of CAT in the homogenate of the lungs and liver of all experimental groups apparently differed in the analysis of rank variations of Kruskal-Wallis test (Table 6). In the blood serum of experimental animals, the level of CAT in all experimental groups did not differ statistically significantly from the control. In lung tissues, the level of CAT was significantly lower in animals of group 2 (29.26%) and group 3 (45.66%) against control values. In liver tissues, the level of CAT was significantly lower in animals of group 2 (38.25%) and group 3 (43.86%) against control values (Table 6).

The CAT in the lungs of rats apparently differed in animals of groups 1 and 3 ($p = 0.02$) and, accordingly, in the liver of rats – in 1 and 3 ($p = 0.03$) and 2 and 3 groups ($p = 0.03$) with the highest value by the combined use of κ -carrageenan and sodium glutamate.

When comparing the enzyme link indicators of the antioxidant defense system (relative to control), the lowest values of the studied parameters were found in 3-D experimental group, while the use of κ -carrageenan caused an increase of SOD and CAT activity with the highest values in liver tissues (Figure 2).

Correlation analysis showed a direct relationship between the activity of SOD and the concentration of TBA in the blood serum and the studied indicators of LPO in the liver of rats injected with κ -carrageenan. In group 2, a negative relationship was established between the activity of CAT and the studied indicators of LPO in the lungs and liver. It is also worth noting the presence of the moderate inverse relationship between SOD activity and the concentration of TBA in the homogenate of the lungs and liver of rats of group 2. The combined use of κ -carrageenan and MSG revealed a negative relationship between the indicators of LPO and enzymes of the antioxidant system in liver tissues, as well as between the activity of CAT and the studied products of LPO in the lungs (Table 7).

Table 5: Indicator of SOD activity in the tissues of rats (con. units/mg of protein) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	31.55 (30.6; 32.18)	18.60 (18.48; 19.13)	21.60 (21.18; 22.53)
Group 1	34.95 (33.78; 36.33)	22.65^ (21.98; 23.43)	30.40^ (29.38; 31.25)
Group 2	27.05 (26.30; 27.45)	14.25^ (13.50; 15.88)	16.15^ (15.78; 16.45)
Group 3	24.65 (23.55; 25.35)	8.15^ (7.8; 8.50)	8.65^ (8.20; 9.10)
Kruskal-Wallis coefficient, p	H = 3.91; $p < 0.05$	H = 6.08*; $p = 0.047$	H = 6.31*; $p = 0.043$

Notes: *Statistically significant results; ^The probability of differences relative to control.

Table 6: Indicator of CAT in the tissues of rats (con. units/mg of protein) under the combined effects of food additives.

Group	Blood	Lungs	Liver
Control	17.55 (16.98; 18.10)	15.55 (15.13; 15.88)	14.25 (13.75; 14.83)
Group 1	18.95 (18.48; 19.58)	16.40 (16.18; 16.88)	16.10 (15.90; 16.23)
Group 2	12.20 (11.80; 12.80)	11.00 (10.55; 11.58)	8.80^ (8.45; 9.05)
Group 3	11.40 (10.68; 11.75)	8.45^ (8.20; 8.83)	8.00^ (7.78; 8.20)
Kruskal-Wallis coefficient, p	H = 4.41; $p < 0.05$	H = 6.31; $p = 0.04^*$	H = 8.03; $p = 0.045^*$

Notes: *Statistically significant results; ^The probability of differences relative to control.

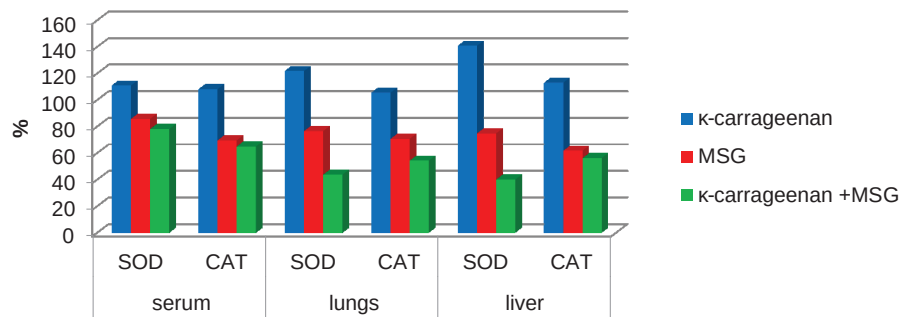


Figure 2: Comparison of indicators of the enzyme link of the antioxidant defense system (%) in the tissues of the body of rats under the combined effects of food additives.

Table 7: Correlation between the indicators of lipid peroxidation and indicators of antioxidant protection of tissues of rats under the combined effects of food additives.

Variables	Group 1		Group 2		Group 3	
	SOD	CAT	SOD	CAT	SOD	CAT
Serum						
DC	r = 0.38; p = 0.07	r = 0.32; p = 0.13	r = -0.31; p = 0.14	r = -0.25; p = 0.23	r = -0.13; p = 0.52	r = -0.11; p = 0.41
TBA	r = 0.42*; p = 0.04	r = 0.40; p = 0.06	r = -0.30; p = 0.15	r = -0.34; p = 0.11	r = -0.27; p = 0.21	r = -0.29; p = 0.17
Lungs						
DC	r = 0.11; p = 0.41	r = 0.13; p = 0.52	r = -0.38; p = 0.07	r = -0.46*; p = 0.02	r = -0.37; p = 0.07	r = -0.48*; p = 0.02
TBA	r = 0.15; p = 0.41	r = 0.18; p = 0.52	r = -0.42*; p = 0.04	r = -0.48*; p = 0.02	r = -0.29; p = 0.17	r = -0.55*; p = 0.005
Liver						
DC	r = 0.55*; p = 0.005	r = 0.39; p = 0.06	r = -0.37; p = 0.07	r = -0.46*; p = 0.02	r = -0.42*; p = 0.04	r = -0.52*; p = 0.01
TBA	r = 0.52*; p = 0.01	r = 0.37; p = 0.07	r = -0.55*; p = 0.005	r = -0.52*; p = 0.01	r = -0.48*; p = 0.02	r = -0.46*; p = 0.02

Note 1: r is the correlation coefficient; p is the level of reliability.

Note 2: *Statistically significant results.

Discussion

Studies over the past 10 years have confirmed that MSG causes disorders such as headache, asthma, diabetes, muscle pain, atrial fibrillation, ischemia, trauma, convulsions, stroke, Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, Parkinson's disease, depression, multiple sclerosis, schizophrenia, obsessive-compulsive disorder, epilepsy, drug addiction, attention deficiency/hyperactivity disorder, fronto-temporal dementia and autism [35–37]. The development of these disorders is

based on typical pathological processes. The universal mechanism that plays a key role in the implementation of the action of most toxic agents is the activation of free radical processes and the development of oxidative stress [38]. The mechanism of cell damage by free radicals consists of their ability to initiate the peroxidation of lipids and proteins, covalently bind to bio macromolecules, and generate reactive forms of oxygen and nitrogen that are highly toxic and capable of initiating cell death [39, 40].

Our results indicate the activation of free radical oxidation and reduced activity of the

enzymatic link of the antioxidant defense system in the blood, lung tissues and liver of rats under intragastric administration of MSG at a dose of 50 mg/kg, which may be associated with direct hyperproduction of oxygen due to the introduction of the studied food additive. It should be noted that the liver has an autonomous system of detoxification of the body, where normally there is a leakage of unpaired electrons, and hence reactive oxygen species. Respiratory and metabolic functions of the lungs are closely related to lipid metabolism, which makes lung tissues prone to damage by various mechanisms, such as lipid peroxidation, oxidative modification of proteins, and DNA damage. This, in turn, can affect the state of phospholipids in the human body and affect the composition of the lung surfactant, which is the largest biological membrane in the human body, which includes saturated and unsaturated fatty acids, where free radical processes are also take place that regulate surfactant properties and support ventilation of lung tissue [41]. In the work of O. Onyema and co-authors is showed that long-term use of MSG causes oxidative stress and increased free radical oxidation of lipids and proteins in the body [42]. Decreased levels of major antioxidant enzymes and increased lipid peroxidation in the kidneys of rats that were consuming MSG have been demonstrated in works of M.V. Paul and co-authors [43] and M. Thomas and co-authors [44]. The authors note that the depletion of antioxidant protection correlates with an increase of LPO. L.P. Gordienko and co-authors found that under conditions of MSG-induced obesity in the salivary glands of experimental rats was significantly increased the content of TBA active products compared to the control. In the tissues of the salivary glands of animals, catalase and SOD activity was significantly reduced relative to control animals [13]. R.Z. Hamza and co-authors investigated that MSG causes an increase of LPO levels in parallel with a significant decrease of SOD activity and CA in testicular tissues [45]. The results of our study can be explained by the data of Tezcan and co-authors [46], who note that TBA is one of the ending products of LPO, and it is formed as a reaction product of cyclooxygenase in the metabolism of prostaglandins, which confirms the presence of oxidative stress in rats treated with MSG. The study of

S. Kumari and co-authors [47] demonstrates that its toxicity is directly related to hyperpolarization of the mitochondrial membrane, increased ROS production, and increased oxygen consumption by mitochondria, mitochondrial dynamic imbalance up to cleavage and activation of autophagy. Regarding changes of SOD and catalase activities under the isolated use of MSG, results are also consistent with the studies of V.V. Bevzo, who showed that a 4-week administration of 3% MSG solution at a rate of 30 mg/kg of body weight of rats reduced the activity of these enzymes in both blood serum and liver homogenate [14]. Another study showed that long-term administration of MSG causes an increase in the generation of ROS by blood neutrophils by 40.3%. Prolonged administration of MSG causes an increase of TBA content by 56.2% in blood serum and 2.1 times in lung homogenate [48, 49]. It should be noted that MSG receptors are present in many tissues of the body, including the brain, heart, lungs, pancreas, where glutamate acts as a neurotransmitter [50]. Consumption of large amounts of MSG can overload MSG receptors in the body and artificially increase the level of free MSG in the body [51].

The results of our study showed a probable increase of lipid peroxidation in parallel with an increase of SOD activity in lung and liver tissues with the use of κ -carrageenan at a dose of 40 mg/kg. Scientific data indicate that under normal digestion, due to acid hydrolysis, κ -carrageenan is broken down into low and high molecular particles that trigger free radical processes in the small intestinal wall at both studied concentrations of κ -carrageenan [52, 53]. The authors note that the use of 1.0% solution of κ -carrageenan is accompanied by the involvement of organs in the pathological process, in particular, the liver and heart, which justifies the generalization of biochemical processes at the body level. Previous studies indicate the involvement of macrophages in the uptake of κ -carrageenan with the formation of heterolysosomes, which leads to the realization of the harmful effects of lysosomal enzymes. On the other hand, macrophages are known to be a source of free radicals [54]. Activation of free radical oxidation processes in liver tissues may be due, according to O.S. Tkachenko and co-authors, as a direct stimulation of the generation of reactive

oxygen species by κ -carrageenan, and indirectly by the stimulating effect of tumor necrosis factor- α , the concentration of which increases with the use of carrageenan [55].

In modern conditions, people consume different food supplements in different combinations and different concentrations every day, which necessitates their combined effect on the body. According to the results of our study, under the combined effect of κ -carrageenan and MSG, lipid peroxidation and depletion of the antioxidant defense system in the lungs and liver increase, with a probable medium-strength relationship between pro- and antioxidants. At present, it is difficult to say what effect the studied food additives have in combination: additive effect or potentiation of effects. On the one hand, both dietary supplements affect oxidative stress; on the other hand, the toxic effects of the studied supplements are also associated with other mechanisms: inflammation under the effect of κ -carrageenan [56] and neurotoxicity under the effect of MSG [57]. However, both with the use of MSG and its combined effect with κ -carrageenan, according to our results, there is a depletion of antioxidant reserves, while with the use of κ -carrageenan, antioxidant activity increases, thereby reducing the negative impact on tissues. Therefore, we consider the dominant negative effect of MSG on the processes of free radical oxidation.

Conclusions

The combined use of a solution of κ -carrageenan and MSG has a negative effect on the processes of free radical oxidation, which is manifested by a statistically significant increase of lipid peroxidation products levels in blood serum, lungs and liver and decreased activity of antioxidant system enzymes in the lungs and liver and in the process significant medium-strength correlations between pro- and antioxidants were established.

Conflict of Interest

The authors declare no conflict of interest.

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Original Research

The current fetal cell-free DNA in the blood of pregnancies without complications and with gestational and diabetes 2 type mellitus

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Received: 13 May 2021 / Accepted: 10 June 2021

Abstract

Background and Aim: Non-invasive prenatal testing (NIPT) has another name for non-invasive prenatal screening (NIPS) – this area of prenatal diagnosis is rapidly developing and using the latest technologies. New generation sequencing for detection of fetal extracellular DNA in maternal plasma or other methods for evaluating extracellular DNA is the basis of NIPT and is used in 80% of cases to detect the main aneuploidies – trisomy 13, 18, 21. This study was aimed at comparing two simplified methods for isolating fetal DNA from peripheral blood, and then comparing the results in pregnant women without complications and pregnancies with one type of complication. **Results:** We tested peripheral blood samples from 64 normal and 29 abnormal pregnancies fetuses. The gestational age had a mean of 21 weeks. Maternal age has a median of 29 years. From 10 mL of maternal blood, we isolated a mean of $10.3 \pm 1.15 \times 10^6$ mononuclear cells. The median number of isolated fetal nucleated red blood cells, corrected for 10 mL blood, was 31.2×10^4 cells in group II 72.4×10^4 – group I after magnetic-activated sorting and 11.7×10^4 cells in group abnormal pregnancy, 29.5×10^4 – normal after hemoglobin enrichment. There is a significant statistical difference between the numbers of total NRBC isolated by the two techniques and research groups ($p < 0.001$). **Conclusion:** Using a modified single-cell – based droplet digital PCR (sc-ddPCR) NIPT, researchers conducted a proof-of-concept study that successfully assessed the genetic information of extremely rare fetal cells in patients with uncomplicated pregnancy and women with gestational diabetes mellitus, as well as in pregnant women with type 2 diabetes complicated by hypertension.

Keywords: cell-free fetal DNA, gestational diabetes, magnetic-activated cell sorting, non-invasive prenatal testing.

Introduction

Non-invasive prenatal testing (NIPT) has another name for non-invasive prenatal screening (NIPS) – this area of prenatal diagnosis is rapidly developing and using the latest

technologies. New generation sequencing for detection of fetal extracellular DNA in maternal plasma or other methods for evaluating extracellular DNA is the basis of NIPT and is used in 80% of cases to detect the main aneuploidies – trisomy 13, 18, 21 [1, 2, 3,4].



There is evidence that the improvement of this technology will soon increase the reliability of detecting micro-deletions/duplications throughout the genome [8], which have been identified as a common cause of a number of human diseases.

Many non-invasive prenatal screening tests today analyze fetal extracellular DNA that is mixed in with large amounts of cell-free DNA in the mother's blood. However, it has long been known that a very small number of fetal or placental cells, estimated at fewer than 10/mL, circulates in pregnant women in the peripheral blood. The currently used NIPS, based on extracellular cfDNA, detect only the fetal fraction, which is about 5–20% of the total DNA, this affects the reliability and specificity of the study. Many authors indicate that the reliability is significantly influenced by such factors: history of malignant neoplasms, chromosomal mosaicism of a pregnant woman, body mass index (BMI), copy number variants (CNV), organ transplantation. When conducting NIPT, the above factors can affect the reliability, which will lead to false-positive or false-negative results [1–4]. When analyzing the literature data, it can be concluded that NIPT is a screening test that requires diagnostic confirmation (by such methods as FISH). However, the determination of methods for isolating extracellular cfDNA for subsequent analysis remains the most actuality.

Although much of our work has utilized a maternal-white-blood-cell-depletion method to enrich for target-cell candidates, we have also collaborated on a positive enrichment strategy using magnetic-activated cell sorting (MACS) [6, 7, 9]. In this research, we have returned to positive selection with MACS.

This study was aimed at comparing two simplified methods for isolating fetal DNA from peripheral blood, and then comparing the results in pregnant women with and without complications.

Materials and Methods

Patients

This study included women who had previously signed an informed consent: with a

singleton pregnancy; undergoing planned prenatal diagnostics and antenatal care. After obtaining informed consent, approved by the Ethics Committee of the Ukrainian Association of Biobanks, 10 mL of peripheral venous blood (which did not exceed the volume of standard studies) was taken into K2 EDTA vacuum units and processed for 1–4 hours. All women underwent an ultrasound examination, the results of which were diagnosed with the normal development of the fetus (absence of structural or chromosomal abnormalities).

Scientists at the Ukraine Association of Biobank and Department of Genetics, Obstetrics, Gynecology and Fetal Medicine Kharkiv Medical Academy of Postgraduate Education (Kharkiv, Ukraine) recruited 64 pregnant women without any obstetrical complications (group I) and abnormalities malformations (group II) as determined by fetal ultrasonography. Group II included 29 women in total: 18 of them had gestational diabetes mellitus and 11 cases with hypertension during pregnancy + type 2 diabetes (Table 1).

Isolation of nucleated cells from the peripheral blood of pregnant women

At the first stage, the samples that arrived at the laboratory for 15 minutes were mixed on a roller, followed by a 1:1 dilution of Dulbecco's phosphate buffered saline (PBS) without Ca and Mg (Biochrom AG), layered on a double density gradient of 1.119 g/mL and 10 mL 1.077 g/mL Percoll (Fluka). At the second stage, the samples

Table 1: The type of pregnant women with pregnancy complications.

The type of pregnancy complications	Group research	The number of cases (n)
Pregnancy-induced hypertension+diabetes 2 type	Group II	11
Gestational diabetes mellitus	Group II	18
Normal	Group I	64

were centrifuged at 500 *g* for 30 minutes at a temperature of -20°C .

The next step was to select the cell ring at the border of the two density gradients. For this, it was taken with a Pasteur pipette and washed twice with Dulbecco's PBS solution at a 1:1 dilution, followed by centrifugation at 500 *g* for 10 minutes. Cellular localization was performed using a density marker – green beads 1.102 g/mL and orange 1.035 g/mL, Sigma.

In the third step, the supernatant was removed and the cells were resuspended in 3 mL buffer at a ratio of 1:10 of the MACS BSA stock solution with autoMACS™ wash solution (buffer B). Cells sorted by magnetic activation sorting (MACS) were resuspended, processed, and stained cells were observed using a fluorescence microscope system (EVOS life, Thermo Scientific, USA).

Separation and generation sequencer of nucleated cells from the maternal blood

While this technology uses a sequencing-by-synthesis method and emulsion PCR (emPCR) similar to other platforms, it differs in that it doesn't use fluorescence or chemiluminescence. Instead, it measures the H^+ ions released during base incorporation (Ion Torrent's, Ion OneTouch 2 System, USA). The Ion OneTouch™ ES employs proven magnetic bead technology to isolate template-positive Ion Sphere™ particles that can be loaded directly onto the ion semiconductor chip—delivering automated, highly reproducible enrichment with every run.

Statistical analysis

The data were expressed as mean $\pm$ standard error of the mean. Statistical differences among the prospective groups and their counterparts were analyzed using one-way analysis of variance (ANOVA) as part of an SPSS software package (v.16.0 for Windows, 2007; SPSS, Inc., Chicago, IL) by a post hoc test followed by Dunnett's for several comparison tests to compare treated groups against respective controls.

Significant differences were indicated by *p* values < 0.05 .

Results and Discussion

Glucose intolerance first diagnosed during pregnancy is gestational diabetes mellitus (GDM) [10]. A diagnosed GDM has a long-term adverse effect on the health of a pregnant woman, increases the risk of adverse outcomes in childbirth, and also predisposes the child to coronary heart disease, obesity, metabolic syndrome, and type 2 diabetes at a later age [10–12].

From the point of view of a number of researchers, fetal nuclear red blood cells (NRBC) are a reliable candidate for analysis, since they are well morphologically differentiated and because they contain a representation of the entire fetal genome and, although they have a limited lifespan, they have specific markers and allow analysis with magnetically activated cell sorting [13–15].

We analyzed and tested peripheral blood samples from 64 pregnant women without pathology and 29 samples from women with abnormal pregnancies. The mean gestational age of all women was 21 weeks (range 12–30). The average age of the pregnant woman was 29 years (range 20–42). As a result of isolation of nucleated cells from 10 mL of peripheral blood of pregnant women, on average, $10.3 \pm 1.15 \times 10^6$ mononuclear cells were sequenced (Figure 1) (mean $\pm$ SE).

We did not find the variations of isolated total NRBC with gestational age like others, using a magnetic-activated cell sorting.

The median number of isolated fetal nucleated red blood cells, corrected for 10 mL blood, was 31.2×10^4 cells in group II 72.4×10^4 – group I after magnetic-activated sorting and 11.7×10^4 cells in group abnormal pregnancy, 29.5×10^4 – normal after hemoglobin enrichment. There is a significant statistical difference between the numbers of total NRBC isolated by the two techniques and research groups ($p < 0.001$) (Figure 2).

The standard protocol for dPCR is designed for the analysis of nanograms of genomic DNA; however, the amount of cfDNA

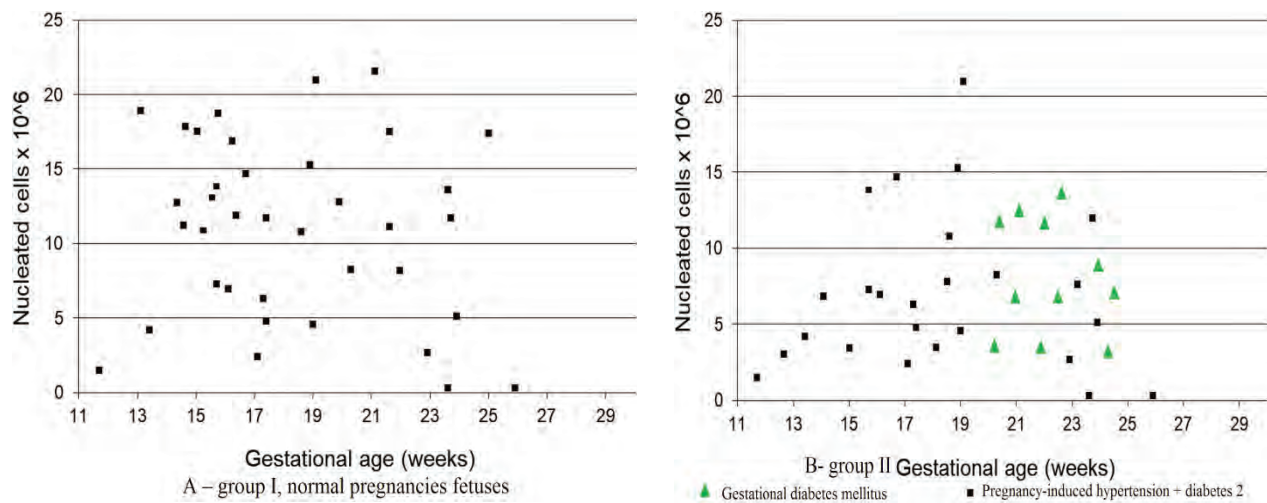


Figure 1: The level of NRBC isolated by centrifugation in the density gradient depending on gestational age (A – group I, B – group II).

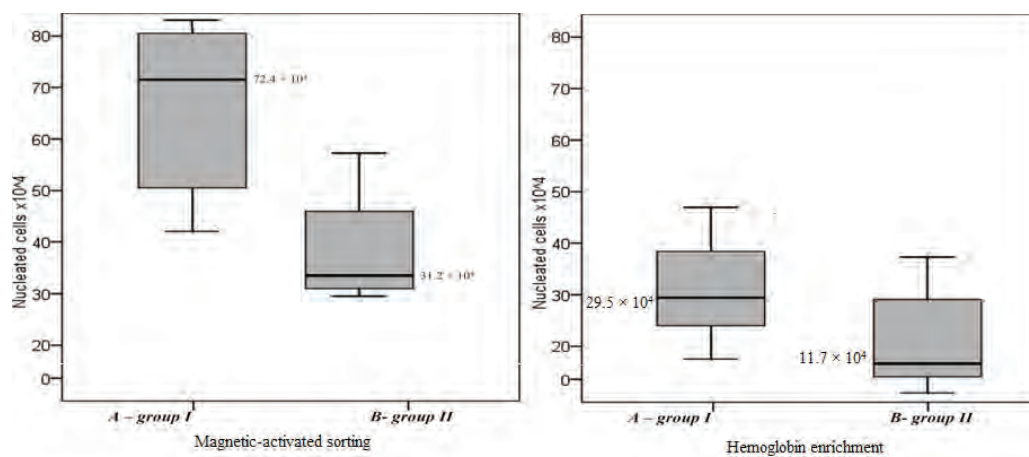


Figure 2: The median number of isolated NRBC, corrected for 10 mL blood in group of abnormal and normal pregnancy.

in plasma samples is much lower. The optimal number of pre-amplified target molecules for dPCR at normal and abnormal pregnancy. The cfDNA was extracted from the plasma mixtures and conducted to pre-amplification PCR. After purification, dilution series from 10^7 to 10^3 molecules per chip of the pre-amplification products were analyzed by dPCR. The analysis of 10^6 , 10^5 , and 10^4 molecules revealed similar results of normal and abnormal pregnancy. In women with hypertension and type 2 diabetes arising during pregnancy, a low level of target/total cells was established and corresponded to 10^7 molecules, a similar trend was observed for the level of antibodies/total cells, which also corresponded to 10^7 molecules. Lower numbers of molecules (12.7×10^4 and 19.7×10^3) in pregnancy-induced hypertension + diabetes 2 type lead to overestimation of

the target/total ratio. The results obtained from 106 molecules were the most robust; thus, 10^6 pre-amplified molecules were used in all pregnancy group dPCR (Table 2).

We found an average of five cells in 10 mL blood for paramagnetic selection, and three cells in 10 mL blood for anti-CD71 and anti-CD14 selection. We evaluated CD14– CD71+ cell quantity and the efficiency of group patient's abnormal and normal pregnancy. A 24-hour incubation in control medium was finally selected as differentiation protocol. Cells became adherent and the expression of recognized macrophage markers, CD71 analyzed by immunofluorescence staining to confirm the monocyte-to-macrophage differentiation in group normal pregnancy, also clearly increased. The expression of CD14, which decreases with macrophage differentiation [19], was also studied

and confirmed the differentiation. Cells were then fixed and immunolabeled for CD14 and CD71 using specific antibodies (green). Nuclei were detected with To-pro3 (blue) (Figure 3).

Table 2: Results from digital PCR analysis in dilution series of pre-amplified cell-free DNA from normal and abnormal pregnancy.

Group	Preamplifier molecules	Target/total, %
Pregnancy-induced hypertension + diabetes 2 type	10 ⁷	5.05 ± 1.33
	10 ⁶	4.27 ± 0.72
	10 ⁵	7.8 ± 0.86
	10 ⁴	12.74 ± 1.15
	10 ³	19.7 ± 0.96
Gestational diabetes mellitus	10 ⁷	5.75 ± 1.4
	10 ⁶	5.1 ± 0.85
	10 ⁵	8.6 ± 1.3
	10 ⁴	14.55 ± 1.55
	10 ³	23.1 ± 1.25
Normal	10 ⁷	6.13 ± 1.1
	10 ⁶	5.3 ± 0.92
	10 ⁵	9.4 ± 1.4
	10 ⁴	16.2 ± 1.4
	10 ³	29.4 ± 1.34

Mean ± SD values from biological triplicates.

The stationary capture of fetal nucleated erythroblasts in the magnetic column through a single separation cycle allowed the elimination of non-specific cells with a 92% efficiency. However, these values are lower than those obtained by Huang et al.

Dragos Nemescu et al. in a similar experiment (median separation efficiency 98.6%, percentiles 20 and 80, 98.1% and 99%, respectively) [16–18]. However, other groups, using a similar method, have obtained significantly more CD71 positive cells (mean of 34–149 × 10⁴ cells/10 mL blood), with a lower depletion rate (94–97%) [14, 15]. Variations in the initial cell number and the different measurement methods (automatic vs. manual) can explain these differences.

Conclusion

Using a modified single-cell-based drop-let digital PCR (sc-ddPCR) NIPT, researchers conducted a proof-of-concept study that successfully assessed the genetic information of extremely rare fetal cells in patients with uncomplicated pregnancy and women with GDM, as well as in pregnant women with type 2 diabetes complicated by hypertension.

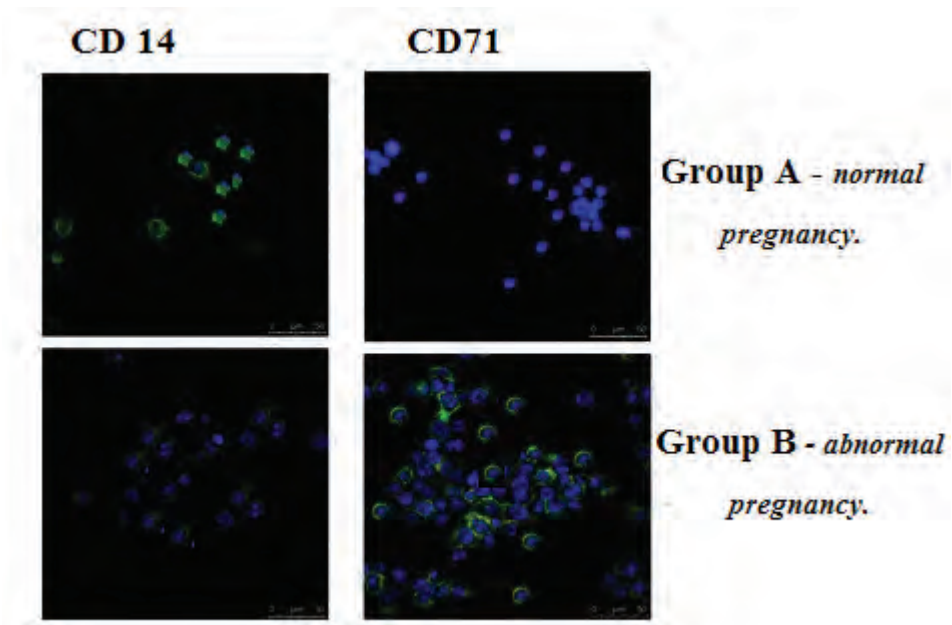


Figure 3: The CD14–, CD71+ cell quantity and the efficiency of group patient’s abnormal and normal pregnancy. The fixed and immune-labeled for CD14 and CD71 using specific antibodies (green).

This modified magnetic-activated cell sorting system makes it possible to directly assess single-cell DNA information from live cells without cell-fixation, cell-staining, and whole genome amplification steps.

The results obtained will allow identifying candidates for participation in early interventional studies and criteria for close monitoring of pregnant women with an increased risk of gestational diabetes mellitus and hypertension associated with pregnancy associated with type 2 diabetes.

Conflict of Interest

The authors declare no conflict of interest.

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Review

Obesogenic behaviors, impact on the general population

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Received: 9 May 2021 / Accepted: 10 June 2021

Abstract

Obesity is a chronic multifactorial, multisystemic disease, responsible for 4.7 million premature deaths annually. The epidemic of obesity is an important challenge for public health all across the world. Now more than ever, obesity affects globally both developing countries and wealthy societies. In Europe, for example, more than half of the population is affected by adiposity. Obesity-related diseases are responsible for the reduction of the life expectancy with almost four years. Rising prevalence of obesity increases the risk of debilitating multimorbidity and mortality. Personal behaviors such as diet, physical activity, screen time, stress levels have an play a key role in the efficiency of individual interventions and comorbidity prognostic. Nowadays, the excessive weight gain at the population level leads to an increasing burden of cardiometabolic comorbidities.

Keywords: adiposity, obesity, metabolism, behavior, diet, physical activity, diseases

Problem Statement

Urbanization and globalization are main contributors to adipogenesis. Obesity defined as excess adiposity for height. Obesity increases the risk of chronic diseases due to metabolic dysfunction [1]. Adiposity is one of the most threatening health issues, affects the quality and the longevity. Nowadays, the modern unhealthy behavior is linked to multimorbidity. Weight gain related to unhealthy lifestyle is one of the biggest killers in the world, leading to a high burden of cardiovascular diseases and type 2 diabetes. According to the World Health Organization (WHO), more than half of all obesity-related non-communicable diseases could be prevented through a healthy lifestyle. The body mass index (BMI) is the gold standard score used in clinical practice to establish the degree of adipose tissue expansion. It is accepted that a BMI higher than 30

kg/m² is usually correlated to a high risk of adiposity-related chronic conditions, end-organ damage, impairment and lowers quality of life through multimorbidity and impairment [2]. Cardiovascular and metabolic diseases are the most common causes of death in developing and developed countries. Guidelines recommend risk assessment in primary care (age, sex, ethnicity, family history, smoking, alcohol consumption, sedentary behavior, increased blood pressure, elevated fasting glucose, increased triglycerides, decreased high-density lipoprotein cholesterol, increased low-density lipoprotein cholesterol, central obesity) and periodic individual screening of blood pressure, heart rate, weight and waist circumference. Romania is the second leading country in Europe in terms of all-causes death[3]. Also, the Romanian population has the lowest life expectancy in Europe, reflecting unappropriated behaviors and socioeconomic issues.



The actual burden of chronic diseases is on the rise mainly due to the most common health risk factors as unhealthy eating, tobacco use, low physical activity and binge drinking. According to WHO, two thirds of the Romanian population is sedentary and almost three fifths of the adults do not consume fruit and vegetables [4]. A range of anthropometric, behavioral and sociodemographic factors are associated with risk factors as unhealthy diet and physical inactivity [3]. Adipocyte expansion is linked to hypercaloric, lipid-rich, carbohydrate-rich meals. Along with the urbanization process, negative lifestyle changes lead to a lower consumption of fruits, vegetables, fiber rich grains and to a lower ability to perform physical activity [5].

Background

The progress in technology and communication engineering, generated an increased screen-based time, which lead to a reduction of daily sports activity at the population level. In Europe, one in three individuals avoids engaging in a physical activity routine. Recent data shows that the amount of time spend sitting also shows 40% [6]. Thus, weight gain trends are increasing due to a lower adipocyte burn rate [4] and lower thermogenesis than in the previous generations. Evidence shows that negative behavioral patterns have been found to lead to a decreased level of the physical activity in the neighborhood and surroundings. Lifestyle interventions and pharmacotherapy centered on reduction of energy intake, increased metabolic rate, lower cardiovascular and metabolic risk are preventing and delaying onset of an array of debilitating conditions such as diabetes, stroke, ischemic heart disease, diabetic neuropathy, osteoarthritis, obstructive sleep apnea and obesity-hypoventilation syndrome. Lifestyle policy centered on appetite, energy intake and proactive environment may lead to positive outcomes in developed countries. There is a need for better health policies centered on lifestyle practices as energy intake control, physical activity and proactive environment [4]. Eating healthy food is an important factor for overall good health. The

reaction to food stimuli along with a baseline physical activity may predict the weight regain after weight loss interventions [7]. A good quality diet consists in a daily intake of fruit and vegetables leading to a better control of the body fat total percentage and a healthy weight range, as a result of high dietary fiber intake [7]. The daily recommended quantity of such products are at least one fruit and one portion of vegetables [8]. It is well-known that cost, taste and food safety are key factors that may improve a health, diet adherence as well as the quality of life. Thus, the degree of knowledge about effective lifestyle actions centered on food quality and baseline exercise lowers weight regain variability after weight loss interventions. The low cost of fast food, the large variety of junk food, along with the big portion size are factors that influence the individual's daily excess calories and eating habits. Also, diets low in fiber, rich in sugar and fats are linked to obesity [9]. Dieting is not enough in order to properly manage the weight issues, individuals must gradually increase the intensity, frequency and timing of physical activity [9]. The impressive progress of technology, communication and entertainment industry decreased physical activity levels [4]. Changes in the built living environment can account for the influences of behavior formation and behavior change. Daily physical activity improves health status and reduces the risk of developing non-communicable diseases [7]. Worldwide, physical inactivity is estimated to be the primary cause of approximately 21–25% of breast and colon cancers, 27% of diabetes and approximately 30% of ischemic heart disease [4]. The recommended amount of cardiorespiratory fitness for a healthy individual per week is at least 150 minutes of moderate physical activity or 76 minutes of high levels of physical activity, in addition to other activities involving movement as active transportation, house chores, gardening and recreational sports activities [9]. Isotonic and isometric muscular contraction (in high and moderate-intensity physical activity) leads to [10]. It is well-known that moderate-to-vigorous exercise improves the quality of life through a better attention, perception, cognition, memory, a higher processing speed, better emotion control, ability to self-monitor personal

behavior, to initiate tasks, to organize daily activities, plan future actions and also a better quality of sleep. Physical activity increases serotonin levels, boosts immune-response to stressors, infections, prevents and controls non-communicable diseases and psychiatric disorders, such as anxiety or depression [11]. Also, daily physical training along with healthy dietary principles, high quality aliments, micronutrient and macronutrient dense meals, boost the metabolic rate. The city planning, the physical activity opportunities (green spaces, built environments for recreation), the public transportation availability level up community's engagement in physical activity [11].

Aims and Research Methods

Data shows that more than three quarters of the disability burden and mortality in the European Region are interconnected with non-communicable diseases, including obesity, cardiovascular diseases and type 2 diabetes [4]. Physical, socioeconomical, educational and environmental factors have a negative impact on behavioral patterns. The aim of the research was to analyze recent data concerning the main behavioral risk factors in the Romanian population. Behavioral risk factors are responsible for more than half of the deaths in the European Region. Romania is one of the countries in the region with the lowest life expectancy. Using the latest available data we analysed the average daily kilocalorie consumption. We included the most common consumed aliments, such as wheat and products, milk, eggs, meat, nuts, fruits, vegetables, oil crops, animal fats, sugar and alcoholic beverages. Also, we evaluated the impact of the most common risk factors on the Romanian population, as well as the health outcomes. We interpreted morbimortality statistics of the most prevalent diseases in Romania. In the analysis, behavioral aspects, physical environment and socioeconomical factors were included. The deadliest diseases in the region were evaluated in correlation to health risk factors[4], such as (dietary saturated fatty acids, trans-fatty acids) and physical inactivity. Results showed

that cardiovascular and metabolic diseases are the most prevalent[4]. Available data shows also that globally, physical inactivity is responsible for almost 21–25% of breast and colon cancers, 27% of diabetes and approximately 30% of ischemic heart disease [4]. A high score of the BMI, unhealthy dietary patterns, low levels of physical activity, lead to a lower quality and shortening of the life. A BMI higher than 30 is closely related to a higher probability of blood pressure oscillations, higher LDL levels and serum glucose levels, leading therefore to an increased cardiovascular and metabolic risk [12]. In order to establish the precise health impact, clinicians use scores and risk charts. Many studies show that a person with obesity has a two-fold higher risk to develop cardiometabolic syndrome. For individuals with morbid obesity, this risk is ten times higher [13]. Cardiovascular diseases, mainly ischemic heart disease and stroke, remain the leading causes of lost years of life [14]. Surprisingly, global estimates show that half of the adult mortality is caused by cardiovascular issues, followed by diabetes [4]. Cardiometabolic risk is linked to traditional bio-clinical risk factors such as hypertension, insulin resistance, dyslipidemia, a proinflammatory profile, the central disposition of the adipose tissue[15]. Thus, behavioral negative factors that decrease metabolism, such as the amount of time spend sitting, the lack of consumption of nutritious dense food play an important part in assessing the probability of cardiovascular or metabolic events. It is well known that adipose tissue dysfunction leads to excessive ectopic accumulation, local inflammation, insulin resistance, lipotoxicity, increases the cardiovascular and metabolic risk [15]. In prolonged negative energy balance conditions, including dietary restrictions and increased energy expansion such as walking between 4000 and 18,000 steps/day, health outcomes are improved, while walking 7500 steps per day boosts cardiovascular function [16].

Cardiometabolic Biochemical Risk Factors

Visceral and ectopic adiposity elevates cardiometabolic risk through cytokines that

mediate inflammation and through adipokines which control glucidic and lipid metabolism [17]. These metabolic disruptions are involved in the development of hypertension, dyslipidemia, hyperglycemia and thrombosis. Also, chronic inflammation of the adipose tissue leads to a lower immune adaptability to cellular stressors [17]. Adiposity is related to a higher adrenergic vascular reactivity, higher cortisol levels, hyperhomocysteinemia, high C reactive protein, low HDL levels, high triglycerides, high plasma levels of fibrinogen, high β -thromboglobulin levels, inactivity of tissular plasminogen and microalbuminuria [17].

Findings

In the next three decades, overweight, obesity and overweight-related diseases might reduce life expectancy by nearly three years. Behavioral factors have a powerful impact on the health outcomes, life expectancy, morbidity and mortality. A 20% reduction of calorie content in energy-dense foods will reduce almost 1.1 million cases of non-communicable diseases and would avoid health costs [1]. Food intake and physical activity are considered key determinants of BMI score and obesity related comorbidities. Healthy diets are five times more expensive than regular food and remain unaffordable for more than 3 billion people in the world, leading to an increasing trend of obesity. For low-income and middle-income individuals, protein-rich foods, dairy, fruits, vegetables are considered high-cost food groups [17].

When purchasing food, individuals living in the Europe are more concerned about taste (45%), food safety (42%), cost (40%) over sustainability concerns, such as food source (34%) and nutrient content (33%) [21]. A healthy and sustainable diet involves eating a balanced diet, rich in fruit and vegetables. Two in five persons living in the European Region do not include in their diet fruit or vegetables. Also, more than half of the population in Europe avoids eating seasonal food products (53%) and home-cooked meals (57%) [21]. Daily intake of high fat products, sugar and salt increase weight gain, overweight and obesity [22].

Although, in Romania, the rates of overweight and obesity are lower than in most countries of the world [22], data shows that the prevalence of obesity increased from 8.3% in 1975 to 22.5 in 2016. Over the past ten years, in Romania the main risk factors were high blood pressure (40%), high cholesterol levels (20%), obesity (20%), physical inactivity (31%), insufficient consumption of fruit and vegetables (29%), smoking (28%) and alcohol consumption (14.4 liters per year) [5]. Romania has the lowest consumption per capita of fruit and vegetables in the European Region. Data shows that two thirds of the Romanian population avoids eating the recommended daily amount of fruits and vegetables [19]. Romania has also the highest rates of avoidable deaths (31.9%) in Europe and more than half of them can be attributed to a lack of effective primary prevention and public health interventions [20]. In Romania, the population neglects behavioral and lifestyle factors that might improve health outcomes [7]. The age-standardized mortality rate is used as an indicator for health systems performance. Both the treatable and preventable mortality indicators for the Romanian population are the highest in Europe. The share of avoidable deaths reached 80.1% of the total deaths registered in the country, compared to the Europe average of 68% [7].

According to the latest INSSE (National Institute of Statistics - Romania) data, the highest obesity rates in Romania are in Mehedinți, Arad, Bihor Countries [1]. As for the calorie intake, the consumption of wheat in Romania per capita is high. In 2018, for example, the daily intake of cereals and other grain products was about 952 kcal.

Between 2014 and 2018, the average consumption of fruits, vegetables and nuts in Romania were lower than the sugar the intake. The data shows a daily consumption of 45 kcal of fruits, 90 kcal of vegetables and 19 kcal of nuts. Otherwise, the daily amount of sugar consumed was about 247 kcal. The daily intake of basic products in Romania is almost one medium egg, meaning 49 kcal/capita/day, 437 kcal/capita/day of milk-based products, 48 kcal/capita/day of oil crops, way lower than the consumption of animal fat 133 kcal/capita/day. [17] More than one third of adults in Romania engaged in episodic heavy alcohol consumption (binge drinking) at

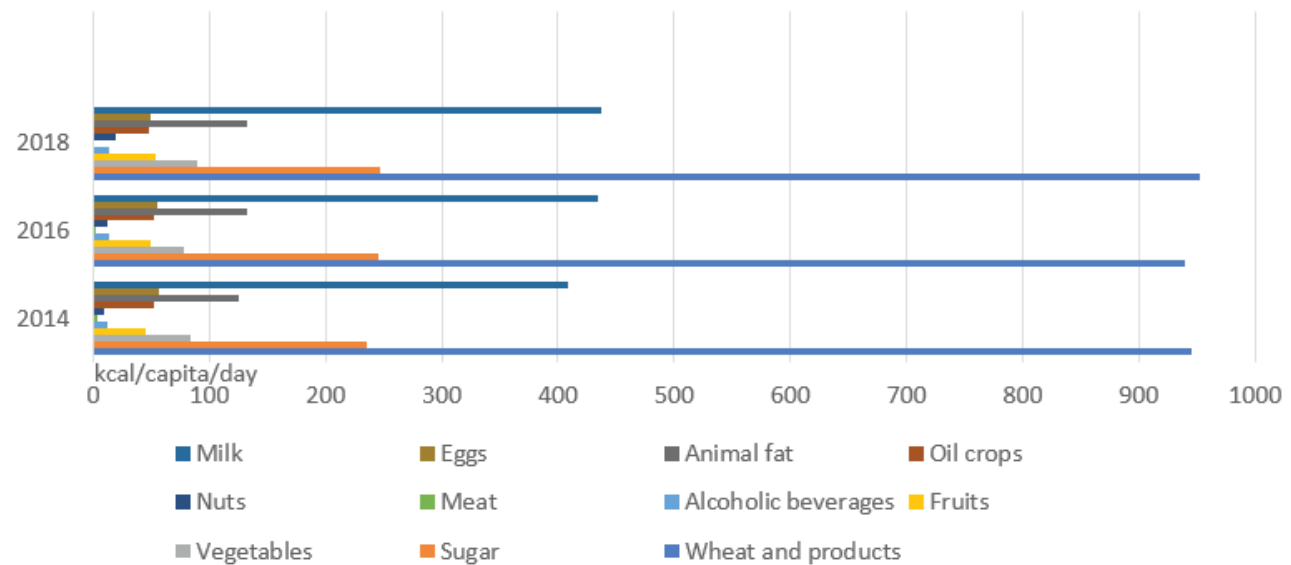


Figure 1: Average daily kilocalorie consumption in Romania (own chart). Data Source: Eurostat data.

least once a month, the second highest rate in the EU (35% compared to 20% on average in the EU). Moreover, there is a strong gender difference, with more than 50% of men reporting having engaged in heavy drinking. Dietary risks include more than ten components, such as low fruit and vegetable consumption, high sugar-sweetened beverage and salt consumption [23]. On the other hand, a Computer Assisted Telephone Interviewing realized on 1030 respondents between 7 and 8 January 2021 showed that more than half of the men refuse to diet (61%). Almost half of the women (45%) were planning to lose weight [23]. Surprisingly, our data shows that the Romanian individuals in the lower socio-economic status are less predisposed to develop overweight and obesity. On the other hand, people who live in cities whose age is more than 51 years are more interested in dieting [23]. According to WHO, only 30% of the Romanian population is practicing sports [4]. Another similar Computer Assisted Telephone Interviewing Survey, realised on 1085 persons living in Romania, between 18 and 21 December 2020 included the analysis of the use of the personal and public transportation. More than half of the participants to the study used public transportation were women (61.0 %) [24]. The collected data shows that almost 70% of the persons aged 18–65 years use more often the public means of transportation. More than half of the individuals aged more than 65 years, transit

the community via local public transportation (51.9 %) [24]. The men (67.4%), as well as the 18–35 years category of individuals (68.8%) and the persons with a high level of education (75.3%) drive more often than the low education category of population. The study showed that individuals with a lower educational achievement travel more often by bus, trolleybus, subway or trains [24]. Also, the same study shows that almost half of the respondents drive. Most of the respondents (67.4%) who drive a car are men with a higher education level. Data also showed that people aged between 18 and 35 years drive more often (68.8%) [24]. Thus, a slightly lower percentage of drivers' age between 36 and 59 years (59.0%), 14.5% of the Romanian individuals who age more than 65 years drive their own car [24].

Strengths and Limitations

Obesity is a progressive, multi-factorial disease, characterized by adipose tissue dysfunction. Lifestyle changes, behavior therapy, individualized pharmacological and surgical interventions have their place, as well as limitations, in the obesity treatment [25]. Lifestyle modifications should be pursued in all patients with obesity before pharmacotherapy and bariatric surgery in order to reduce cardiometabolic risk. Added value of this study: In Romania, the diet is

based on wheat-based food products, milk products, sugar and animal fat. This dietary pattern decreases cellular adaptability, decreases metabolic flexibility, leading in time to an increased risk of insulin resistance, obesity and cardiovascular diseases. We estimated that the daily intake of these aliments reduces cellular thermogenesis, decreases mitochondrial function leading to low adenosine triphosphate levels, low metabolization of glucidic and lipidic elements. We searched for solutions to increase biodisponibility of cellular adenosine triphosphate through positive behavioral and dietary patterns at the population level. The effective control of neurochemical substrates of appetite, stress, blood sugar, emotions, stress reactions are tied to metabolism and weight loss interventions. Populational behavioral modifications, meaning the reduction of psychophysical stressors, the avoidance of the most important health risk factors, lead in time to better compensatory responses,

an effective metabolic energy balance, a better quality of life and a healthy weight.

Limitations: There is a lack of populational studies concerning the relationship between behavioral, environmental factors and the ascending obesity related burden of disease. Future research should also identify the correlation genetic polymorphism, adipose percentage and disposition, dose-response exercise activity levels, alongwith effective dietary patterns, in order to reduce biochemical markers and the early onset of diseases.

Discussions

Obesity reflects unhealthy behaviors, socioeconomic inequalities at the population level, as well as socioeconomic deficiencies. in health systems. Reducing the obesity burden requires complex approaches that adapt individual interventions to the environmental and societal changes [25]. Lifestyle and behavioral interventions aimed to provide a better health status, promoting the reduction of sugar, salt, caloric intake and increasing energy expenditure. Medical professionals have to be involved in counseling patients, encouraging behavioral

modifications in patients at intermediate and high risk of developing cardiometabolic diseases. Sustained individual efforts are needed to maintain cardiovascular benefits of weight loss interventions or pharmacotherapy.

Obesity-related non-communicable diseases might be prevented through the reinforcement of local strategies. Therefore, the burden of disease and the public care health costs can be considerably reduced [24]. Diet adherence, the portion size, pricing, the availability of nutritional dense food products are involved the rising prevalence of burden of obesity-related diseases [24]. Population strategies that address qualitative food and beverages, active living promotion and safe living environments may provide a healthier and performance of the workforce [4]. In order to reshape the context of health policy, a multilevel approach is needed. Recomendations in local guidelines, mass-media interventions, high-quality screening interventions, medical counseling (healthy lifestyle, reduction of risk factors, BMI/ body fat percentage/central adiposity) have proven positive outcomes over the past decades in the reduction of non-communicable diseases in many developed countries. Local national screening programmes of the patients at risk, along with clinical cardiometabolic risk estimations and evaluations, help identify diseases at an early stage and prevent future medical negative events. The reinforcement of strategies addressing sustainable health and socio-economic development goals is needed in order to prevent multimorbidity [4]. Also, sustainable active transportation, walkable and bikeable neighborhoods, are very effective in reducing the impact of obesity-related acute and chronic diseases.

Conclusions

Tackling the obesity pandemics is a challenge for public health services all around the world due to it's powerful impact on acute and chronic diseases, such as cerebrovascular, cardiometabolic, peripheric vascular disease, thereby contributing to a decline in both quality of life and life expectancy. The interaction

between genetic and environmental factors lead to a low energy expenditure and increase the prevalence of obesity [26]. In people with obesity, positive outcomes in weight reduction is a challenge because of a lower compliance to lifestyle and behavioral interventions, pharmacotherapy or bariatric surgery. While diet, exercise, caloric restriction, smoking cessation, may improve cardiometabolic scores, health risks often remain the same for several months due to ineffective neurohormonal modulation [25]. In many countries of the world, despite the efforts aimed to improve prevention of obesity-related chronic diseases through governmental campaigns, obesity-related noncommunicable diseases remain a public health concern, affecting the entire population. Looking ahead to the future generations, actions must be taken at micro and macro levels in order to raise awareness of the obesity issue and to reduce the threat for the public health systems. State legislators and policymakers need to focus their attention on family-friendly healthy life strategies, centered on the control of main health risk factors and screening.

Conflict of Interest

The authors declare no conflict of interest.

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Review

Nutritional assessment of patients with type 2 diabetes and chronic kidney disease: a review

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Received: 8 May 2021 / Accepted: 10 June 2021

Abstract

It is a well-known fact that the prevalence of type 2 diabetes (T2DM) increases each year. It has a complex etiology that includes irreversible risk factors such as age, genetics, race, and ethnicity, as well as reversible risk factors such as diet, physical activity, and smoking. The goals of this review are to look at various studies that investigate the paths to assess the nutritional status of patients with T2DM who are also linked to chronic diseases. In patients with type 2 diabetes, malnutrition has been recognized as a serious health problem, mainly in hospitalized conditions. Therefore, we gathered information that helps the physician look into the patient's nutritional status in order to have a better outcome.

Keywords: chronic disease, clinic and para-clinic evaluation, malnutrition

Worldwide, the prevalence of diabetes is rising, with the largest increase occurring in low- and middle-income countries. In most developed countries, type 2 diabetes is currently the main cause of end-stage renal disease, and it also causes a large number of cardiovascular diseases. In countries with weaker economies, type 2 diabetes is rapidly replacing infectious diseases as the main cause of kidney disease and is competing for scarce medical resources [1]. The risk of chronic kidney disease (CKD) for people with diabetes is nearly 2 times higher than for people without diabetes. The odds ratio of CKD varies from 1.3 to 4.6, depending on the world, and this risk is exacerbated by the presence of high blood pressure [1].

As with other diseases, the clinical definition of CKD in diabetic patients is persistent albuminuria (albumin to creatinine ratio [ACR] ≥ 30 mg/g for at least 3 months) and/or persistent underestimated glomerular filtration rate (eGFR ≤ 60 mL/min/1.73 m²), regardless of the cause.

The nutritional assessment is a complex process through which the information (clinical, laboratory, para-clinical, etc.) can be collected, classified and integrated. This is a phased process, starting with more complex procedures from basic nutritional screening to determining body composition and diagnosing abnormal metabolism of macro- or micronutrients [2]. Nutritional risk screening is a simple and fast first-line tool used to detect patients at risk of malnutrition and should be systematically performed in hospitalized patients [3].

Disorders of nutritional status are very common, with at least 40% of the world's population being affected by some form of malnutrition (malnutrition or over nutrition). Supernutrition through its most common clinical form, abdominal obesity, is a defining criterion of the metabolic syndrome, having a great impact on cardiovascular risk, often encountered in patients with type 2 diabetes [2, 4].



In addition, the predictive power of mortality may be high; for example, higher BMI and serum lipid levels associated with poor prognosis in the general population can predict improved survival of CKD, a phenomenon known as reverse epidemiology [5].

On the other hand, malnutrition (especially proteic) is a common complication of most chronic diseases, which strongly predicts repeated/long-term hospitalization, adverse evolution and high mortality. Therefore, for patients with chronic diseases, adequate nutritional treatment through nutritional means (nutrition medical intervention), especially risk detection/quantification and its subclinical manifestations are the basic goals [2]. Under nutrition has been frequently reported in patients on admission to hospital. Since this situation is not always detected in time, screening of nutritional risks on admission has been widely advocated [6].

According to this definition, malnutrition can be referred to as both over- and undernourished. Although the incidence of over nutrition among the elderly in developing countries is increasing due to the poor development of the elderly and changes in their eating habits, in general, under nutrition is still a major concern. Therefore, in this review, unless otherwise stated, malnutrition refers to under nutrition unless otherwise specified [7].

Malnutrition is a very common problem, affecting approximately 30–50% of hospitalized patients. Hospital malnutrition is associated with an increase in morbidity and mortality, a higher rate of readmission, and the need for rehabilitation support after discharge. Therefore, medical and social costs are higher. In different studies in Spain, it is estimated that among the elderly population, the prevalence of malnutrition in hospitals is between 12.5% and 78.9% [8]. It is believed that the aging process is one of the causes of malnutrition, which will eventually affect the nutritional status of the elderly. The American Society for Parenteral and Enteral Nutrition (ASPEN) defines malnutrition as acute, sub-acute or chronic nutritional conditions, in which varying degrees of over nutrition or under nutrition (with or without inflammatory activity) lead to changes in body composition and weakened functions [7].

Malnutrition is an independent risk factor that can negatively affect the patient's clinical outcome, quality of life, physical function and autonomy. In order to provide timely and appropriate nutritional support, it is important to detect patients at risk of malnutrition or malnutrition early [3].

The pathogenesis of malnutrition in chronic kidney disease is complex and involves a variety of pathophysiological changes, including decreased appetite and nutrient intake, hormonal imbalance, metabolic imbalance, inflammation, increased catabolism, and abnormalities related to dialysis. Malnutrition increases the risk of morbidity, mortality and overall disease burden in these patients [9].

Anorexia is common in CKD patients and may be caused by appetite (appetite stimulation) and anorexia (appetite inhibition), accumulation of metabolic waste products, kidney failure, peculiar smell and the effect of drugs on taste buds. The cumulative effect of these factors leads to a reduction in nutritional intake. The result of the chronic inflammatory state in CKD is an increase in resting energy expenditure, which promotes protein catabolism and reduces anabolism [9].

The methods used to assess nutritional status are: clinical examination, which includes the anamnesis (including specific means of investigating dietary intake) and a thorough physical examination, including anthropometric measurements and the search for specific signs of certain nutritional deficiencies; biochemical data, which have the role of identifying/quantifying major nutritional deficiencies and to objectify to a certain extent the intake of certain nutrients; para-clinical data, especially procedures for determining body composition (with varying degrees of accuracy) [2].

Clinical Exam

Nutritional anamnesis

The physiological and pathological history of the patient constitute the first chapter in the nutritional anamnesis and address especially the presence of chronic or acute diseases that can influence the nutritional status, information about the

chronic use of medication recommended by the doctor or self-administered (including legal/illegal drug use), vitamins, nutritional supplements, natural remedies, etc. The patient should also be asked about food allergies or known intolerances (e.g. lactose, gluten, etc.) [2].

Family history

They include two types of information: the existence of blood relatives of genetically transmitted diseases (such diseases have a family agglomeration without an absolute inheritance, for example, hypertension, type 2 diabetes, etc.) and diseases due to contagion in a familiar environment, a common way of life or exposure to the same toxins [10]. Another type of information needed in this history is family eating habits: if meals are shared, if eaten at a restaurant, gastronomy (frying fatty foods, using animal fats for cooking, eating bread, etc.); the amount and type of alcohol consumed in common at meals, etc. [2].

Socio-professional background

Includes a series of data that can be very relevant. They are varied enough to be grouped into several categories: professional, social, geographical and lifestyle of the patient (toxic consumption, eating habits (may be related to religion, occupation or place of residence) and extra-occupational physical exertion are known disease or health factors [2, 10]. Another category of data characterizes the patient's ability to understand his or her health problems as well as to adhere to a long-term TMN plan: functional and memory capacity, level of education, type of emotional response to finding out his medical condition (chronic/incurable disease, need for insulin therapy, likelihood of initiating dialysis, etc.) [2].

Dietary history. Usual intake (dietary history). The method of estimating the usual intake, also called dietary history, is a retrospective method by which the patient is asked to describe his daily "usual" intake qualitatively and quantitatively. From a technical point of view, the examiner may provide the patient with a

questionnaire to complete (possibly as long as he or she is waiting for consultation), which will list the main food groups, along with a description of a "portion" of the food, accompanied by questions such as: "How many such portions are usually consumed daily/weekly?" [2].

Food frequency questionnaire. This is another retrospective method, which complements the dietary history and refers to the frequency with which the patient consumes the main food groups. This procedure usually insists on certain foods that have clinical significance, such as fruits, vegetables, meat, eggs or dairy products. Currently, relatively simple tools have been created that integrate the procedures of the dietary anamnesis in order to be used quickly as a screening in daily practice; they can be addressed to patients, such as the Rapid Eating Assessment for Patients (REAP) form [11], or they can be used by healthcare professionals who do not have special clinical nutrition training, such as the WAVE (Weight, Activity, Variety) guide and excess [2, 12].

Subjective global assessment (SGA) is a tool used by healthcare providers to assess nutritional status and help predict nutrition-related clinical outcomes (such as postoperative infection and/or mortality). This tool has many advantages in clinical and research environments: it is cheap, rapid to conduct; it can be effectively used by providers from different disciplines, such as nursing, nutritionist, and physician; and it is found to be reproducible in some studies, effective and reliable. Due to its advantages, SGA has been recommended by the National Kidney Foundation (NKF) Kidney Disease/Dialysis Outcomes and Quality Initiative (K/DOQI) for nutritional assessment of adult dialysis populations [13].

In the past few decades, a variety of scoring and screening systems have been established, which can be used in various clinical settings and patient populations (inpatients, communities, geriatrics, etc.). Screening should be carried out within the first 24–48 hours after admission, after which it should be screened regularly (for example once a week) in order to quickly and accurately identify those who should be referred to nutrition experts (for example nutritionists, expert clinicians) personal further evaluation. Nutrition screening should include dynamic

parameters rather than static parameters, such as recent weight loss, current body mass index (BMI), recent food intake, and disease severity [3].

One of the most commonly used nutritional risk screening tools in hospitals around the world is NRS-2002. NRS-2002 was developed by Kondrup *et al.* and is intended to be a generic tool in the hospital setting – that is, useful in detecting the majority of patients who would benefit from nutritional therapy [14].

The Global Subjective Nutritional Assessment (SGA) is a practical, non-invasive and inexpensive composite tool that is widely used in clinical practice [5].

Use the 4-point SGA scale to assess nutritional status. The scale consists of 6 components: three are based on the patient's weight loss history, the incidence of anorexia and vomiting, and three are based on the physician's degree of muscle wasting, the presence of edema and subcutaneous loss as mentioned before. PEW_{SGA} is defined as an SGA score > 1 , and a score of 1 indicates that the nutritional status is normal. In several studies, SGA was found to be a reliable tool for assessing nutritional status, and is related to clinical features, anthropometrics, and nutritional biomarkers also shown in this study. However, due to its subjectivity, people question the effectiveness of SGA as a nutritional marker [5].

Cooper *et al.* who compared SGA with total body nitrogen as the gold standard for protein deposits in 76 dialysis patients reported that although SGA could differentiate severely malnourished patients from those with normal nutrition, it could not predict the degree of malnutrition [15].

Jones *et al.* using a compound nutritional score derived from SGA, body mass index, percentage of reference weight, triceps fold thickness, arm circumference, and serum albumin found that SGA could not reliably identify hemodialysis patients with abnormal nutrition [16]. On the other hand, Steiber *et al.* found that 7-point SGA is a reliable and valid tool for nutritional assessment in hemodialysis adults [17], and Cuppari *et al.* comparing SGA with anthropometric parameters concluded that 7-point SGA is a reliable tool for the evaluation of PEW in patients with chronic kidney disease without dialysis [18].

The most widely used are weight loss in the past 6 months, body mass index, subjective self-assessment of similar scales (SGA is used for subjective global assessment) [19], nutritional risk index NRI ($15.9 \times \text{serum albumin concentration (g/dL)} + 41.7 \times (\text{current weight/normal weight}) \times 100$), [3] nutritional risk score (NRS) [6], general screening tool for malnutrition (MUST) and plasma albumin concentration [20].

Practically, European guidelines propose the following criteria to define severe malnutrition, in which preoperative management must be mandatory (recommendation level: A):

- loss of more than 10% of body weight in less than 6 months, or weight loss of more than 5% for a month;
- and/or body mass index $< 18.5 \text{ kg/m}^2$;
- and/or SGA score of C grade;
- and/or serum albumin concentration $< 30 \text{ g/L}$ [20].

Physical Exam

Determining and interpreting the usual anthropometric indices

Anthropometric measurements are considered to be part of the physical examination, are relatively simple and can be important in assessing nutritional status, especially in patients with chronic diseases, which can cause significant changes in body composition through edema or loss of muscle mass. In order to be able to compare evolving anthropometric indices, they must be measured by standardized (reproducible) procedures. The main anthropometric indices used for the diagnosis and monitoring of obesity (supernutrition), in patients with type 2 diabetes, are the BMI and AC. Another significant parameter especially for patients with chronic diseases (in which there is a risk of global and/or protein malnutrition) for those associated with CKD is weight change over a standard period, usually 6 months, expressed as a percentage of weight. initial (or “usual” weight) of the patient [2].

Alternative equations that use limb length instead of height may be valuable for

epidemiological and clinical work, because there was no predictive equation before. Five equations that combine triceps-skinfold thickness with other anthropometrics explain >80% (male) and >77% (female) variance. The most powerful prediction is based on waist circumference and triceps skinfold thickness. Skinfold thickness measurements continue to provide good predictions of average body density, but there are significant deviations in extreme body fat and age. The most reliable prediction with the least deviation is the waist circumference adjusted for age [21].

Another important parameter is the percentage change in weight, especially for monitoring the nutritional status of patients at risk of malnutrition, such as those with chronic diseases. This index is expressed as a percentage of the patient's initial weight, or of the patient's "usual" weight. If it exceeds 10%, it is severe, and weight loss is common in the elderly, in addition to the association with CKD, and the mechanisms by which chronic diseases can induce weight loss are more often it is about reducing food intake, by decreasing appetite or for objective reasons: edentation, difficulty chewing/swallowing, pain caused by food intake, vomiting, etc. Another category of mechanisms are social: family/social disinsertion, poverty, food procurement difficulties [2].

There are many links between obesity and chronic kidney disease. There are many pathophysiological pathways (such as chronic inflammation, increased oxidative stress) and pathophysiological pathways (such as chronic inflammation, increased oxidative stress and insulin resistance, hypertension, and dyslipidemia) between obesity and chronic kidney disease [22].

The data obtained through the physical examination are valuable because they guide the nutritionist towards certain specific deficiencies, but usually require confirmation through laboratory and/or para-clinical examinations.

Biological evaluation

Laboratory and para-clinical examinations are routinely used in the practice of assessing nutritional status, having the advantage that

they are objective and require only a small amount of patient cooperation. There are currently a large number of biochemical determinations that have nutritional significance; they can be classified into: tests to assess energy and protein intake, biomarkers of inflammatory syndrome, methods for estimating body composition.

Assessment of energy and protein intake/status

These biochemical tests evaluate (usually indirectly) both the dietary intake and the protein compartments of the body. These two aspects cannot be separated, because: an adequate energy intake is essential for maintaining the optimal protein status, otherwise the proteins will be used energetically (inefficiently) for energy production by gluconeogenesis; an optimal quantitative and qualitative protein intake (especially through the intake of essential amino acids) is crucial for the structural and functional integrity of protein compartments, the two major protein compartments of the body – the somatic, represented by skeletal muscle, and visceral, represented by "pool" of albumin – have different diagnostic and prognostic significance, so they should be investigated separately [2].

The rate of hepatic synthesis of most proteins used as nutritional biomarkers decreases under the effect of inflammatory cytokines, which is why they are also called "acute phase negative reagents" (albumin, transferrin); there are also proteins whose plasma levels correlate positively with the markers of the syndrome. Inflammatory, called "positive phase positive reactants" (ferritin, fibrinogen). Due to the difficulty of discriminating between the effects of the two mechanisms (intake deficit, respectively inflammation), the values of protein biomarkers should be interpreted only together with the other determinations (inflammatory markers) and in the context of clinical data [2].

Albumin

The albumin concentration in the body is affected by albumin synthesis, degradation

and distribution. Although albumin has the highest concentration of any protein in the vascular system, more than 60% of the protein exists in the extravascular space. During the period of protein consumption caused by major surgery or infection, albumin from the extravascular pool can be mobilized, so the serum albumin concentration may not drop for a long time. The rate of albumin denaturation is proportional to the size of the extravascular pool, which makes the concentration in the serum relatively constant. The long biological half-life of albumin (20 days) only allows changes in serum concentration after chronic malnutrition. Since there are many mechanisms that may cause the albumin concentration to decrease, the isolated serum albumin level may have limited value in assessing the liver synthesis rate of critically ill patients [23].

Serum albumin is not a good indicator of short-term protein and energy deficiency. However, albumin levels are a good indicator of chronic deficiency. Traditionally, albumin has been used to help determine two important nutritional states. First, it helps to recognize chronic protein deficiency when sufficient non-protein calories are consumed, leading to significant hypoalbuminemia. This may be due to the net loss of albumin in the intravascular and extravascular pools. Second, the albumin concentration may help determine a disease called marasmus. This is caused by insufficient calories rather than insufficient protein, so in marasmus, serum albumin levels remain normal, but body weight has dropped considerably [23]. Studies have classified different levels of malnutrition by using albumin levels. Serum albumin levels of 35 g/L or higher are considered normal. Albumin levels of 30–35 g/L indicate mild malnutrition; 25–30 g/L indicates moderate malnutrition; <25 g/L indicates severe malnutrition. Although hypoalbuminemia is common in most patients with diabetic nephropathy, its relationship to the severity and progression of diabetic nephropathy remains largely unknown.

The severity of hypoalbuminemia was significantly associated with an adverse renal outcome, independent of clinical and

histopathological features. A study of 188 patients with type 2 diabetes and diabetic nephropathy reported that people with a lower than normal albumin level showed a 7.37-fold higher risk of progression to chronic end-stage renal disease [24].

Pre-albumin

Determining the level of pre-albumin, a hepatic protein, is a sensitive and cost-effective method for assessing the severity of the disease caused by malnutrition in patients with critical or chronic diseases. Pre-albumin levels have been shown to be correlated with patient prognosis and are an accurate predictor of patient recovery. In high-risk patients, pre-albumin levels measured twice a week during hospitalization can alert doctors of a decline in nutritional status, improve patient prognosis, and shorten hospital stays in an economy that is increasingly concerned about costs. Zinc deficiency may reduce pre-albumin levels, but vitamin deficiency does not [25].

Transferrin

Transferrin is a monomeric glycoprotein found in the biological fluids of invertebrates and vertebrates, and has the property of reversibly binding iron [26]. The main site of transferrin synthesis (but not the only one) is also the liver; the total pool is only 5 g, and the plasma half-life is 8–10 days, which is an advantage compared to albuminemia as a nutritional marker [2]. Current research shows that people with serum transferrin saturation greater than 55% have an increased risk of all-cause death [27]. In addition to its traditional role in iron metabolism, transferrin also acts as a growth factor [28].

Retinol-binding protein

Retinol-binding protein consists of 181 amino acid residues. It has been used to monitor

short-term changes in nutritional status. Its usefulness as a metabolic marker is based on its 12-hour biological half-life and small body pool size. As a polypeptide chain, retinol-binding protein strongly interacts with plasma transthyretin, and circulates in the plasma as a 1:1 mol/L transthyretin-retinol-binding protein complex. Due to its low molecular weight, only the free form of retinol-binding protein which has no affinity for transthyretin can undergo unhindered glomerular filtration. Then, it is reabsorbed by the tubule cells and catabolized there. This may explain the extremely high serum levels of retinol-binding protein in advanced chronic renal insufficiency [23].

Retinol-binding protein (RBP)-4 is a recently discovered adipocytokine associated with insulin resistance. Compared with healthy subjects, the level of RBP-4 in diabetic patients was significantly higher. RBP-4 is significantly positively correlated with triglycerides, systolic blood pressure and urine albumin excretion, and significantly negatively correlated with high-density lipoprotein cholesterol and blood flow-mediated vasodilation [29]. The results are consistent with these findings and may indicate that the degree of obesity (reflected by BMI) is the main determinant of RBP-4 levels [29].

Serum creatinine

Serum creatinine is the most commonly used biomarker of renal function. In the steady state, serum creatinine can be used to evaluate the glomerular filtration rate by adjusting the kidney disease diet (MDRD) or the Chronic Kidney Disease Epidemiology Collaborative Research (CKD-EPI) formula. Serum creatinine is not only considered to be one of the main criteria for the acute kidney assessment of homeostasis and CKD, it also impairs it [30].

The concentration of creatine and creatine phosphate is highest in the skeletal muscle of mammals. Therefore, under steady-state conditions, especially when on a vegetarian diet, the main source of creatinine is skeletal muscle [30].

In healthy individuals, the kidney is the main way to eliminate creatinine. Creatinine has a low molecular weight and does not bind to albumin. [30] Therefore, it is filtered freely at the level of the glomerulus. Creatinine does not have a specific biological function; it is continuously released from muscle cells and excreted by the kidneys being reabsorbed only to a small extent [31].

Other para-clinical investigations

The most accurate estimation of the body composition is of great importance for the evaluation of the nutritional status, because the relative changes of the body compartments significantly influence the diagnostic value of the other nutritional parameters.

Body composition can be assessed by direct and indirect methods. Direct methods address molecular or cellular levels, are precise, but usually invasive and laborious. In practice these methods are used for research purposes [2].

In the early days, body composition analysis was based on anthropometry, which evaluated skinfold thickness (estimating the amount of subcutaneous fat) and/or around the mid-arm or thigh (as a measure of skeletal muscle mass). This method is extended to the evaluation of various parts of the human body through reliable and effective body density measurement of underwater weighing. More advanced methods include dilution technology (D_2O to evaluate whole body water; NaBr to evaluate extracellular water), dual X-ray absorption method (DXA; measurement of bone mineral content, lean soft tissue and fat mass) [32]. MRI or computed tomography (CT; based on ionizing radiation and X-ray attenuation) are used to accurately assess the entire body and regional organ (i.e. skeletal muscle, brain and visceral organs) and tissue masses. In addition to MRI, magnetic resonance spectroscopy measures ectopic fats (e.g. liver, muscle, and pancreas fat). More recently, quantitative (without imaging) magnetic resonance imaging has been introduced to assess adipose tissue (and total body water) with high accuracy [32].

There is currently no universally accepted or comprehensively validated approach to assessing a patient's nutritional status. Moreover, clinical trials have repeatedly shown that no clinical, physical or laboratory examination can adequately predict nutritional risk. It is important for doctors to know both objective and subjective measures of basic body needs and functional capacity [33].

Due to their global ability to assess nutritional needs, clinical assessment systems have gained popularity in clinical practice. However, each has its own limitations, stressing the importance of the need for further evaluation of the effectiveness of these instruments [6]. When used properly, these tools lead to the early identification of patients at risk and allow for rapid intervention, which not only provides better care but also reduces costs [34].

Malnutrition continues to be an independent risk factor that negatively influences patients' clinical outcomes, quality of life, body function and autonomy. However, when equipped with the right clinical tools, the doctor is more likely to provide safe and effective nutritional intervention and ultimately lead to improved clinical outcomes.

Conflict of Interest

The authors declare no conflict of interest.

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Review

Diabetic tongue – a review

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Received: 16 February 2021 / Accepted: 31 March 2021

Abstract

Diabetes mellitus is a long standing illness, presenting with severe complications directly proportional to the degree of hypoglycemia affecting all age group of people. Among all the organs of the body, oral manifestations are mostly the initial complication and could even promise an early diagnosis of the disease. Xerostomia due to tumefaction of salivary gland, burning sensation of oral cavity, recurrent ulcers, angular chelitis, oral lichen planus, infections and periodontal disease are all the commonest oral abnormalities detected in the patient with diabetic mellitus. However, there is very minimal literature regarding the tongue lesions associated with diabetes mellitus which are often neglected. Hence, this paper aims to review the literature regarding the tongue lesions and complications associated with diabetic mellitus and possible future prospects.

Keywords: candidiasis, diabetes mellitus, glossitis, oral manifestations, tongue

Introduction

Diabetes mellitus is a metabolic condition with multiple etiological factors resulting in insulin deficiency or tissue resistance to insulin [1]. American Association of Diabetes in 1977 classified diabetes based on the etiological factors as type 1 (juvenile diabetic) and type 2 (acquired diabetic) diabetes mellitus. Type 1 diabetic mellitus is caused due to the blockade of insulin due to pancreatic beta cell destruction which are not caused by viral or autoimmune origin and common among 1st and 2nd decade of life. Type 2 diabetes arise as a result of alteration in both molecular and cellular level of insulin receptor [2].

Among type 1 and 2 diabetes mellitus, type 2 is most common which precedes with the symptoms such as polyuria, polydipsia, loss of body weight is the most common form of diabetes mellitus and has symptoms that commonly

indicate hyperglycemia are polyuria, polydipsia, weight loss, fatigue, weakness, blurry vision, frequent skin infections, and slow healing of skin lesions after minor trauma [3].

A set of microvascular and macrovascular complications are the substantial cause of mortality and morbidity in diabetes mellitus and may further increase the risk of blindness, kidney failure, myocardial infarction and stroke. Diabetes mellitus shows complicated pathophysiology and has a considerable heterogeneity within the diabetic populations [4].

Oral Manifestation of Diabetic Mellitus

Besides many other pathophysiological changes, diabetics increase the risk of oral manifestations such as periodontitis which are deliberated as the 6th manifestation of diabetic



mellitus. Xerostomia due to tumefaction of salivary gland, burning sensation of oral cavity, recurrent ulcers, angular cheilitis, oral lichen planus, infections and periodontal disease are all the commonest oral abnormalities detected in the patient with diabetic mellitus. Also, there is a decrease in response to infections caused by bacteria, fungal and viral in uncontrolled diabetes patients as a result of hyperglycemia and ketoacidosis. On the other hand, controlled diabetes patients are at low risk though they have good control of glucose level as they are under proper medication and controlled diet [2].

Among the oral manifestations in patient with diabetes mellitus, tongue lesion which is often neglected deserves a special mention. Glossitis, atrophic tongue lesions, median rhomboid glossitis, geographic tongue, coated and fissured tongue, glossodynia and burning mouth sensation, and of taste sensation are the reported abnormalities of tongue manifested with higher incidence among diabetic patients [5, 6]. The intensity of above-mentioned abnormalities are frequently proportional to the hyperglycemic duration and rate [7].

Glossitis

Glossitis is one of the inflammatory manifestations found in diabetes and are associated with stomatitis, skin rashes, loss of weight, diarrhea, decreased level of hemoglobin and plasma amino acid level have been reported to be found in patients with glucagonoma associated with diabetes mellitus [5].

Central atrophic glossitis

Central atrophic glossitis or median rhomboid glossitis of the tongue is probably a form of candidiasis as illustrated by Budtz-Jorgensen in 1972 and Cooke in 1975. Long standing central papillary atrophy of the tongue are found to be a common manifestation of the tongue which arise as a result of predisposing factor such as microangiopathy which is a frequent findings seen in patients with diabetic mellitus [8] (Figure 1).



Figure 1: A 32-year-old male, who was known case of diabetes mellitus showing a central area papillary atrophy along the central longitudinal fissure of the tongue.

Candidiasis of Tongue

Candidiasis in the oral cavity are the earliest and prevalent signs among one fourth of the diabetic population [9]. However, *Candida* is a normal microbial flora usually present in the oral cavity has some triggering factors such as immune dysfunction, hyperglycemia, and acid production can promote candidal infection in diabetes. Elevated salivary glucose levels and higher candidal colony-forming units in saliva can also stimulate the candidal production and increase the incidence of candidiasis in diabetic patients [10]. Atrophy of the filiform papillae leads to atrophic tongue or smooth tongue which favors a fine environment for the conidial growth [11]. A 3/5th of the candidial glossitis which is also termed as acute atrophic glossitis has a pre-disposing factor such as diabetic mellitus which can also be associated with consumption of board spectrum antibiotics and corticosteroid inhalation [12, 13]. Denture induced stomatitis, angular cheilitis and median rhomboid glossitis which precedes with multiple fungal and bacterial etiology are also associated with candidal lesions [14] (Figure 2).

Oral Hairy Leukoplakia

Oral hairy leukoplakia (OHL) is a white lesion caused by Epstein Barr virus (EBV). OHL



Figure 2: A 51-year-old female, who was a known case of diabetes mellitus showing curdy white scrapable patches on the dorsum of the tongue, a PAP smear of the tongue revealed candidal hyphae confirming candidiasis.

occurs in the lateral border of the tongue and are often associated with severe immunodeficiency conditions [15]. There are certain OHL cases in patients associated with minor immunosuppression and also in immunocompetent individuals are also reported in the literature. As far as diabetics are concern, incidence of OHL are due to deficient PMNs leukocyte chemotaxis and abnormal phagocytic action by macrophages [16].

Geographic Tongue

Geographic tongue or benign migratory glossitis (BMG) is an asymptomatic inflammatory condition which exclusively affects dorsum of the tongue [17]. Pathophysiology of geographic tongue in diabetic mellitus may be due to delayed healing and slow tissue repair as a result of hypoxia and oral microvascular disease in patients with diabetic mellitus. Clinically the lesion may appear as single or multiple lesion which is insidious in nature and may present with non-nucleated patches which are denuded of keratin, thus gives a reddish appearance, the adjacent side with a white to cream colored thick margins [18, 19]. In the literature, it has been mentioned that BMG has a genetic pre-disposition exhibiting polygenetic inheritance and

increased incidence of tissue type HLA-B15 has also been reported by mark and trait which is more prevalent among type 1 diabetic mellitus population [20]. Bastos et al. found in a study with 146 type 2 diabetic patients with the prevalence of geographic tongue with type 2 diabetic mellitus about 5.4% higher than that of the control group which had an incidence of 0.9% [21]. A cross-sectional study conducted by Saini et al. in 420 diabetic mellitus patients and a control group has shown that patients with diabetic mellitus have shown the incidence of geographic tongue [21]. Another investigation done with 391 type 2 diabetes mellitus patients with oral lesions by Al-Maweri et al. has also shown that there is higher prevalence of BMG than that of the control group [22] (Figure 2).

Fissured Tongue

Fissured tongue is a developmental or genetic based abnormality of the tongue manifested by abundant furrows or grooves on the dorsal surface of the tongue which is usually asymptomatic, except when there is collection of debris or food in the furrows which may cause discomfort or pain and has a possible chances for infection [23–25]. Fissured tongue with generalized complication associated with double fissure running longitudinally along the dorsum of the tongue, has been reported to occur more



Figure 3: A 29-year-old female patient, who was a known case of diabetic mellitus presented with an irregular smooth erythematous patches on the dorsum of the tongue.



Figure 4: A 40-year-old female, who was a known case of uncontrolled diabetes showing fissures and grooves in the anterior 2/3 rd of the dorsum of tongue.

frequently in patients with type 1 diabetic mellitus [26] (Figure 4).

Altered Taste Sensation

Altered taste sensation or taste dysfunction have many pre-disposing factor in which endocrine and metabolic disorders were proposed as the major etiological factor. Other than that, salivary gland disorder may also imply altered taste sensation or may elevate the detection thresholds [27, 28]. Uncontrolled diabetic patient has reported more incidence of taste dysfunction than the controlled diabetic patient or healthy individuals [29]. Taste dysfunction may further lead to poor glycemic condition due to lack of diet control and has high threshold in the patients suffering from neuropathy [30].

Conclusion

Patient with diabetic mellitus disorder is prone to greater alteration in the oral cavity especially tongue shows a popular incidence among the diabetic population which are often neglected by the clinician. There is a clear-cut exposure of diabetic associated with tongue disorders or abnormalities which may cause candida infection, atrophic glossitis, atrophic glossitis with candida infection, oral hairy leukoplakia,

geographic tongue, fissured tongue and taste dysfunction even though diabetics has a major association periodontal and gingival complications.

Conflict of Interest

The authors declare no conflict of interest.

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Review

The effect of anti-diabetic drugs on the musculoskeletal system in women

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Received: 5 April 2021 / Accepted: 4 June 2021

Abstract

Diabetes mellitus is a chronic endocrine disorder caused by absolute or relative lack of insulin, which subsequently leads to hormonal and metabolic changes in the body. Since patients receive continuous long-term anti-diabetic treatment, there was a chance to analyze the effect of anti-diabetic drugs on bone tissue in type 2 DM (T2DM) patients. The results of the studies indicate that the bone metabolism is the most adversely affected by treatment with thiazolidinediones and canagliflozin from the group sodium-glucose co-transporter-2 inhibitors, while dipeptidyl peptidase-4 (DPP-4) inhibitors and insulin have a positive effect on it, and, finally, glucagon-like peptide-1, biguanides and sulfonylureas have rather a slight impact on the bone tissue.

Keywords: DPP-4 inhibitors, insulin, metformine, osteoporosis, SGLT2, thiazolidinediones.

Introduction

Diabetes mellitus (DM) is one of the most common human diseases and one of the three pathologies often causing early disability and high mortality. Chronic hyperglycemia have an adverse effect on the cardiovascular, urine, nervous and other human body systems [1].

IDF Diabetes Atlas (2019) counts around 463 million people suffering from DM worldwide. One of the rapidly growing complications of diabetes is a disorder of phosphorus-calcium metabolism and bone condition, manifested by early osteoporosis [2]. Recent studies have shown that the impaired bone structure integrity in patients with T2DM may be associated with hypoglycemic drugs [3].

Thiazolidinediones

The thiazolidinediones, oral hypoglycemic drugs include rosiglitazone and pioglitazone.

These medications improve insulin sensitivity and are administered for T2DM treatment [4]. However, the results of the clinical and experimental studies show that thiazolidinediones adversely impact the bone structure [5–7]. ADOPT studies have shown that peri- and post-menopausal women taking rosiglitazone, experienced a higher risk of fractures. Based on the studies it was found that an increased risk of fractures in women is not associated with the difference in peri- and post-menopausal estrogen level [8]. The study of sex differences in the side effects of thiazolidinedione therapy have found that women were twice likely to have an increased risk of fractures [9]. The results of another randomized placebo-controlled study have demonstrated that post-menopausal women with no T2DM and no osteoporosis, after 14 weeks of rosiglitazone therapy, experienced a significant decrease in the level of bone formation markers in the acetabulofemoral joint [10]. Besides, one more study data have shown that despite high bone mineral density, patients with T2DM have an increased risk



of fracture from thiazolidinedione therapy due to impaired stem cell differentiation and effects on osteoblastogenesis [11].

SGLT2i

Sodium-glucose co-transporter-2 inhibitors (SGLT2i) are a new class of drugs used in the complex treatment of T2DM. Their main mechanism lies in the effect on the excretory renal function, causing increased excretion of glucose in the urine, thereby reducing blood glucose levels. [12]. One among the major negative effects caused by SGLT2i, there are genitourinary infections and bone fractures when treated with canagliflozin [13]. The effect of SGLT2i on calcium-phosphorus metabolism and bone tissue in T2DM patients have not been fully investigated yet [7, 5, 14]. Till now, CANVAS clinical study have demonstrated an increased incidence of bone fractures with canagliflozin, which raises the level of bone resorption markers due to weight loss. It raises the level of beta-carboxy-telopeptide, a fragment of type I collagen, composing 90% of bone tissue. Therefore, it can be assumed that an increase in telopeptide levels during treatment with canagliflozin may lead to osteoporotic changes [15, 16]. Some studies also indicate that the canagliflozin reduces estradiol levels in women [17].

Though not all SGLT2i medications have a proven negative effect on the bone matrix, still some studies have shown the correlation of dapagliflozin and empagliflozin treatment and increased level of bone metabolism markers [18–20].

Dipeptidyl peptidase-4 (DPP-4) inhibitors

The class of dipeptidyl peptidase-4 inhibitors (DPP-4i) is associated with less risk of fractures compared to thiazolidinediones and sulfonylureas [21]. Even in the case of long-term use, the risk of fractures is low according to a retrospective cohort study [22].

On the contrary, DPP-4i can improve bone metabolism. In an animal study [23], sitagliptin contributed to the improvement of bone mineral

density while affecting the intestinal hormones GIP (glucose-dependent insulintropic polypeptide), which is involved in bone homeostasis and increases intracellular calcium, enhances collagen and alkaline phosphatase syntheses [24, 25].

Other researches in T2DM patients treated with DPP-4i have shown a low-risk of upper limb fractures [26]. I Barchetta study demonstrates a positive effect of DPP-4i on the vitamin D level in patients with T2DM, which enables to consider its positive effect on bone metabolism as well [27].

Insulin

It is known that insulin drugs should be used with caution to avoid hypoglycemia [19, 20, 28]. An increased incidence of bone fracture in DM patients taking insulin is because these patients usually have a long history of diabetes, they experience more diabetic complications and a much higher risk of hypoglycemia, associated with a higher frequency of falls [29]. Meanwhile, insulin remains the drug of choice for DM patients with existing limb fractures for better glycemic control [19]. Also, insulin preparations affect osteoblast differentiation and proliferation, which in turn produce osteocalcin [28]. The study have shown the osteocalcin effect (namely of its two forms – carboxylated and non-carboxylated) on the β -cells of the pancreas, in terms of improvement of insulin sensitivity and β -cell function [30].

Sulfonylureas

A class of sulfonylurea drugs can also lead to hypoglycemia, especially in elderly patients [5]. Hypoglycemic conditions are accompanied by the increased risk of falls and, consequently, fractures, but it is not the result of a direct impact of medications on bone resorption [31].

Study results show that this class of drugs, similar to insulin, contributes to bone health since they stimulate insulin secretion, which in turn promotes osteoblast differentiation, as described above [28]. One research has

also shown a beneficial effect of glimepiride on bone tissue formation while activating phosphoinositide 3-kinases and v-akt murine thymoma viral oncogene homolog, which improves osteoblast differentiation [32].

Biguanides

A class of biguanides, especially metformin, has a direct osteogenic effect on bones. It influences osteoblast differentiation and bone matrix synthesis, meanwhile promoting osteoblast proliferation through AMP-activated protein kinase (AMPK) [33–35].

Therefore, it can confirm the osteoanabolic properties of metformin [5].

GLP-1 ra

Glucagon-like peptide-1 receptor agonists (GLP-1 ra) play a part in increasing insulin secretion due to incretin hormone function in response to food intake, which helps to suppress glucagon secretion [5]. Experimental studies found that native glucagon-like peptide 1 does not have a direct influence on osteoblasts, but affects osteogenic differentiation indirectly while being present in bone marrow stromal cells [36].

Some study demonstrated that liraglutide significantly reduces the risk of bone fractures, whereas exenatide treatment was associated with an increased risk of accidental bone fractures [37]. A randomized cohort study, based on the effect of liraglutide on bone mass in overweight women and controls, also proved its benefit. The results showed that in the group of women who took liraglutide, the bone mass increased by 16% [38]. A cohort study revealed that the use of GLP-1 ra is not associated with a reduced risk of bone fractures compared with other anti-hyperglycemic drugs [39].

One of the side effects of GLP-1 ra treatment can be nausea, vomiting, and diarrhea, these are likely to lead to malabsorption of minerals and nutrients, which adversely affects bone physiology. [40].

It also should be reminded and emphasized to diabetic patients that aerobic exercises

have a positive contribution to bone metabolism. This is evidenced by studies focused on comparing changes in arm strength and bone metabolism after 6 months in non-insulin-dependent patients. Training on aerobic exercises on the treadmill helps to improve markers of bone metabolism and grip strength in patients with diabetes [41].

Conclusions

The constant use of anti-diabetic drugs can influence the musculoskeletal system. Thus, despite the high hypoglycemic efficiency, the thiazolidinediones cause disorders in stem cell differentiation, especially adversely affecting women, due to their age-related estrogen decrease. According to the latest studies, the group of SGLT2i also harm bone tissue. Though it's only concerning canagliflozin, taking which increases the frequency of fractures, whereas dapagliflozin and empagliflozin do not cause such an effect. Based on the studies, insulin and sulfonylureas have a good influence on calcium metabolism. The main disadvantage associated with these drugs is the risk of hypoglycemia, which can lead to falls and fractures. Osteogenic agents include biguanides, GLP-1 ra, and DPP-4i indirectly act on bone metabolism through bone marrow stromal cells. The biguanide group takes part in the osteoblastic process by osteoblast differentiation, DPP-4i improve metabolism in bone tissue itself, and GLP-1 ra, although indirectly, acts on bone metabolism through bone marrow stromal cells.

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Pentru detalii suplimentare, consultați Rezumatul Caracteristicilor Produsului, scanând codul QR alăturat sau prin intermediul reprezentantului local Eli Lilly.



Toujeo (insulină glargin 300 unități/ml) - Informații esențiale din RCP

Prezentare: Toujeo 300 unități/ml SoloStar soluție injectabilă în stilou injector (pen) preumplut. Toujeo 300 unități/ml DoubleStar soluție injectabilă în stilou injector (pen) preumplut. 1 ml soluție conține insulină glargin 300 unități. Fiecare stilou injector (pen) SoloStar conține soluție injectabilă 1,5 ml, echivalent cu 450 unități. Fiecare stilou injector (pen) DoubleStar conține soluție injectabilă 3 ml, echivalent cu 900 unități. **Indicații terapeutice:** Tratamentul diabetului zaharat la adulți, adolescenți și copii începând cu vârsta de 6 ani. **Doze și mod de administrare:** Toujeo este o insulină bazală, care se administrează o dată pe zi, oricând în timpul zilei, de preferat la aceeași oră în fiecare zi. Când este necesar, pacienții își pot administra Toujeo cu cel mult 3 ore înainte de sau la maximum 3 ore după momentul obișnuit de administrare. Schema de administrare a dozei (doza și momentul administrării) trebuie ajustată în funcție de răspunsul individual. La pacienții cu diabet zaharat de tip 1, Toujeo trebuie utilizat o dată pe zi și trebuie asociat cu o insulină cu acțiune de scurtă durată/rapidă, pentru a asigura necesarul prandial de insulină. La pacienții cu diabet zaharat de tip 2, doza inițială recomandată pe zi este de 0,2 unități/kg. Toujeo poate fi administrat și în asociere cu alte medicamente antidiabetice. **Schimbarea tratamentului:** *Schimbarea tratamentului între insulină glargin 100 unități/ml și Toujeo:* Insulina glargin 100 unități/ml și Toujeo nu sunt bioechivalente și nu sunt interschimbabile direct. În cazul schimbării tratamentului de la insulină glargin 100 unități/ml la Toujeo, aceasta se poate efectua unitate la unitate, dar poate fi necesară o doză mai mare de Toujeo (cu aproximativ 10-18%) pentru a atinge valorile glicemiei din intervalul țintă. În cazul schimbării tratamentului de la Toujeo la insulină glargin 100 unități/ml, doza trebuie scăzută (cu aproximativ 20%), pentru a reduce riscul de hipoglicemie. *Schimbarea tratamentului de la alte insuline bazale la Toujeo:* În cazul schimbării tratamentului de la o schemă de tratament care conține o insulină cu acțiune de durată intermediară sau de lungă durată la o schemă de tratament cu Toujeo, poate fi necesară o modificare a dozei de insulină bazală, precum și ajustarea tratamentului antidiabetic asociat (doza și momentul administrării pentru insulinele regular asociate sau pentru analogii de insulină cu acțiune rapidă asociați sau doza medicamentelor antidiabetice non-insulinice). Schimbarea tratamentului de la insuline bazale, cu administrare o dată pe zi, la Toujeo administrat o dată pe zi poate fi efectuată unitate la unitate, pe baza dozei anterioare de insulină bazală. În cazul schimbării tratamentului de la insuline bazale cu administrare de două ori pe zi, la Toujeo administrat o dată pe zi, doza inițială recomandată pentru Toujeo este de 80% din doza zilnică totală de insulină bazală a cărei administrare se întrerupe. Pacienții tratați cu doze mari de insulină din cauza prezenței anticorpilor anti-insulină umană pot avea un răspuns îmbunătățit la insulină în cazul administrării Toujeo. În timpul schimbării tratamentului și, ulterior, pe parcursul săptămânilor inițiale, se recomandă supraveghere medicală cu monitorizare metabolică strictă. **Pentru toate detaliile privind schimbarea tratamentului, citiți RCP complet.** **Grupe speciale de pacienți:** Toujeo poate fi utilizat la vârstnici, la pacienți cu insuficiență renală și la pacienți cu insuficiență hepatică. La pacienți cu insuficiență renală și la pacienți cu insuficiență hepatică, necesarul de insulină poate fi diminuat. Toujeo poate fi utilizat la adolescenți și copii începând cu vârsta de 6 ani, pe baza aceluiași principii ca în cazul pacienților adulți. Atunci când se schimbă tratamentul de la insulină bazală la Toujeo, scăderea dozelor de insulină bazală și a bolusurilor de insulină trebuie evaluată în mod individual, pentru a reduce la minimum riscul de hipoglicemie. Siguranța și eficacitatea Toujeo la copii cu vârsta sub 6 ani nu au fost stabilite. **Mod de administrare:** Se administrează numai pe cale subcutanată. Locurile de administrare a injecției din cadrul unei regiuni selectate pentru administrare trebuie alternate de la o injecție la alta pentru a reduce riscul de lipodistrofie și de amiloidoză cutanată. Toujeo nu trebuie administrat intravenos sau în pompe de perfuzie pentru insulină. Atunci când se trece de la Toujeo SoloStar la Toujeo DoubleStar, dacă doza anterioară a pacientului a fost un număr impar (de exemplu 23 unități), atunci doza trebuie crescută sau scăzută cu 1 unitate (de exemplu la 24 unități sau 22 unități). Stiloul injector (pen-ul) preumplut Toujeo DoubleStar este recomandat la pacienții care necesită cel puțin 20 unități pe zi. Toujeo nu trebuie extras cu seringă din cartușul stiloului injector (pen-ului) preumplut Toujeo SoloStar sau al stiloului injector (pen-ului) preumplut Toujeo DoubleStar, deoarece poate rezulta supradozaj sever. Pentru detalii privind administrarea, citiți RCP complet. **Contraindicații:** hipersensibilitate la substanța activă sau la oricare dintre excipienți. **Atenționări și precauții:** Pentru a avea sub control trasabilitatea medicamentelor biologice, numele și numărul lotului medicamentului administrat trebuie înregistrate cu atenție. Toujeo nu este insulina de elecție pentru tratamentul cetoacidozei diabetice. Pacienții trebuie instruiți să alterneze continuu locurile de injectare, pentru a reduce riscul de apariție a lipodistrofiei și amiloidozei cutanate. Există un posibil risc de absorbție întârziată a insulinei sau de reglare insuficientă a glicemiei în urma injectării insulinei în locuri unde au apărut aceste reacții. Schimbarea bruscă a locului de injectare cu o zonă neafectată duce la hipoglicemie. Se recomandă monitorizarea glicemiei după schimbarea locului de injectare și se poate avea în vedere ajustarea dozei de medicament antidiabetic. **Hipoglicemia:** Se recomandă prudență deosebită și sporirea supravegherii glicemiei la pacienții în cazul cărora episoadele hipoglicemice pot avea o relevanță clinică particulară, cum sunt pacienții cu stenoză semnificativă a arterelor coronare sau a vaselor sanguine cerebrale (risc de complicații cardiace sau cerebrale ale hipoglicemiei), precum și la pacienții cu retinopatie proliferativă, mai ales dacă nu au fost tratați prin fotocoagulare (risc de amauroză tranzitorie consecutivă hipoglicemiei). Pacienții trebuie atenționați cu privire la circumstanțele în care simptomele de avertizare a hipoglicemiei sunt diminuate. Efectul prelungit al insulinei glargin poate întârzia remiterea hipoglicemiei. **Afecțiuni intercurrente:** necesită intensificarea supravegherii metabolice. **Anticorpi anti-insulină:** Administrarea insulinei poate determina formarea de anticorpi anti-insulină. În cazuri rare, prezența acestor anticorpi anti-insulină poate face necesară ajustarea dozei de insulină pentru a corecta tendința la hiper- sau hipoglicemie. **Asocierea Toujeo cu pioglitazonă:** Au fost raportate cazuri de insuficiență cardiacă în cazul utilizării pioglitazonei în asociere cu insulină, mai ales la pacienții cu factori de risc pentru dezvoltarea insuficienței cardiace. Acest lucru trebuie avut în vedere dacă se ia în considerare administrarea Toujeo în asociere cu pioglitazonă. Dacă este utilizată asocierea, pacienții trebuie supravegheați pentru identificarea de semne și simptome ale insuficienței cardiace, creștere în greutate și edeme. Administrarea pioglitazonei trebuie întreruptă la apariția oricărei deteriorări a simptomatologiei cardiace. **Prevenirea erorilor de medicație:** Înainte de administrarea fiecărei injecții, trebuie verificată întotdeauna eticheta insulinei. **Interacțiuni:** Substanțele care pot intensifica (și crește susceptibilitatea la hipoglicemie) sau scădea efectul de scădere a glicemiei sunt detaliate în RCP complet. **Fertilitatea, sarcina și alăptarea:** Nu există experiență clinică privind utilizarea Toujeo la femeile gravide. Pentru insulina glargin nu sunt disponibile date clinice din studii controlate privind utilizarea sa la femeile gravide. Conform unui număr mare de date privind femeile gravide nu s-au evidențiat reacții adverse asupra sarcinii specifice insulinei glargin și nici efecte malformative sau efecte toxice feto/neo-natale ale insulinei glargin. Utilizarea Toujeo poate fi luată în considerare în timpul sarcinii, dacă este necesar din punct de vedere clinic. Nu se cunoaște dacă insulina glargin se excretă în laptele uman. Nu se anticipează apariția de efecte metabolice ale insulinei glargin ingerate asupra nou-născuților/sugarilor alăptați, deoarece insulina glargin este descompusă în aminoacizi la nivelul tractului gastro-intestinal. **Efecte asupra capacității de a conduce vehicule:** Capacitatea de concentrare și de reacție a pacientului poate fi afectată din cauza hipoglicemiei, hiperglicemiei sau, de exemplu, din cauza tulburărilor vizuale. Pacienții trebuie atenționați să își ia toate măsurile de precauție pentru a evita hipoglicemia în timp ce conduc vehicule. **Reacții adverse: Foarte frecvente:** Hipoglicemia. Hipoglicemia poate să apară dacă doza de insulină este prea mare în raport cu necesarul de insulină. Episoadele severe de hipoglicemie, mai ales dacă sunt recurente, pot determina leziuni neurologice. Este posibil ca episoadele hipoglicemice prelungite sau severe să aibă potențial letal. Frecvente: lipohipertrofie, reacții la locul de injectare care includ eritem, durere, prurit, urticarie, edem sau inflamație. **Cu frecvență necunoscută:** amiloidoză cutanată. Lipodistrofia și amiloidoza cutanată pot întârzia absorbția insulinei. Pentru toate reacțiile adverse, citiți RCP complet. La copiii cu vârsta sub 6 ani, nu sunt disponibile date privind siguranța provenite din studii clinice. Profesioniștii din domeniul sănătății sunt rugați să raporteze orice reacții adverse pe www.anm.ro/Raportează o reacție adversă. **Supradozaj:** Episoadele ușoare de hipoglicemie pot fi tratate, de obicei, prin administrarea orală de glucide. Episoadele mai severe pot fi tratate prin administrare de glucagon intramuscular/subcutanat sau de soluție concentrată de glucoză intravenos. Pot fi necesare aportul susținut de glucide și menținerea sub observație a pacientului, deoarece hipoglicemia poate să reapară după o ameliorare clinică aparentă. **DAPP:** Sanofi-Aventis Deutschland GmbH **Mod de eliberare:** prescripție medicală PRF. **Text revizuit la data de:** februarie 2021, bazat pe RCP revizuit în iulie 2020. **Pentru informații complete de prescriere, citiți Rezumatul caracteristicilor produsului.**

▼ Acest medicament face obiectul unei monitorizări suplimentare. Acest lucru vă permite identificarea rapidă de noi informații referitoare la siguranță. Profesioniștii din domeniul sănătății sunt rugați să raporteze orice reacții adverse suspectate.

Suliqua (insulină glargin 100 unități/ml / lixisenatidă): Informații esențiale din RCP

Prezentare: Suliqua 100 unități/ml + 50 micrograme/ml soluție injectabilă în stilou injector (pen) preumplut. Fiecare stilou injector (pen) preumplut conține insulină glargin 300 unități și lixisenatidă 150 micrograme în 3 ml soluție. Suliqua 100 unități/ml + 33 micrograme/ml soluție injectabilă în stilou injector (pen) preumplut. Fiecare stilou injector (pen) preumplut conține insulină glargin 300 unități și lixisenatidă 100 micrograme în 3 ml soluție. Fiecare ml conține metacrezol 2,7 miligrame. **Indicații terapeutice:** Suliqua este indicat la adulți pentru tratamentul diabetului zaharat de tip 2 insuficient controlat, pentru a îmbunătăți controlul glicemic, ca adjuvant dietei și exercițiului fizic, adăugat la metformină administrată în asociere cu sau fără inhibitori ai co-transportorului 2 de sodiu glucoză (SGLT-2). **Doze și mod de administrare:** Suliqua este disponibil sub formă de două stilouri injectoare (pen-uri); Suliqua 100 unități/ml + 50 micrograme/ml stilou injector (pen) preumplut eliberează doze în trepte cuprinse între 10-40 unități insulină glargin, în combinație cu 5-20 µg lixisenatidă (pen-ul Suliqua (10-40)). Suliqua 100 unități/ml + 33 micrograme/ml stilou injector (pen) preumplut eliberează doze în trepte cuprinse între 30-60 unități insulină glargin, în combinație cu 10-20 µg lixisenatidă (pen-ul Suliqua (30-60)). Pentru a evita erorile de medicație, medicul prescriptor trebuie să se asigure că sunt menționate în prescripție concentrația corectă și numărul corect de trepte de dozare. Doza trebuie stabilită în mod individual, pe baza răspunsului clinic, și se ajustează treptat, în funcție de necesarul de insulină al pacientului. **Doza inițială:** Tratamentul cu insulina bazală sau cu agonistul receptorului pentru peptidul 1 asemănător glucagonului (glucagon like peptide-1 (GLP-1)) sau cu un medicament antidiabetic oral, altul decât metformina și inhibitorii SGLT-2, trebuie întrerupt înainte de inițierea administrării de Suliqua. Doza inițială de Suliqua se bazează pe tratamentul antidiabetic anterior și pe recomandarea de a nu depăși doza inițială recomandată pentru lixisenatidă de 10 µg:

		Tratament anterior		
		Pacienți netratați cu insulină (Tratament antidiabetic oral sau cu un agonist al receptorului GLP-1)	Insulină glargin (100 unități/ml)** ≥20 și <30 unități	Insulină glargin (100 unități/ml)** ≥30 și ≤60 unități
Doza inițială și stiloul injector (pen-ul)	Stiloul injector (pen-ul) Suliqua (10-40)	10 trepte de dozare (10 unități/5 µg)*	20 trepte de dozare (20 unități/10 µg)*	
	Stiloul injector (pen-ul) Suliqua (30-60)			30 trepte de dozare (30 unități/10 µg)*

* unități insulină glargin (100 unități/ml)/µg lixisenatidă

** Dacă se utilizează o insulină bazală diferită: Pentru insulina bazală administrată de două ori pe zi sau pentru insulina glargin (300 unități/ml), doza totală zilnică utilizată anterior trebuie scăzută cu 20% pentru a selecta doza inițială de Suliqua. Pentru orice altă insulină bazală, trebuie aplicată aceeași regulă ca în cazul insulinei glargin (100 unități/ml). Doza zilnică maximă este de 60 unități de insulină glargin și 20 µg lixisenatidă, ceea ce corespunde la 60 trepte de dozare. Suliqua trebuie injectat o dată pe zi, în ora de dimineața a unei mese. Este preferabil ca injecția să fie efectuată în fiecare zi înainte de aceeași masă, după ce a fost aleasă cea mai convenabilă masă. **Ajustarea dozei:** Doza de Suliqua se stabilește în conformitate cu necesarul de insulină al fiecărui pacient în parte (pentru detalii, vezi pct. 4.2 din RCP complet). **Grupe speciale de pacienți:** **Vârstnici** (≥65 ani) Suliqua poate fi utilizat. Doza trebuie ajustată în mod individual, pe baza monitorizării glicemiei. Deteriorarea progresivă a funcției renale poate duce la scăderea constantă a necesarului de insulină. **Insuficiență renală:** Experiența terapeutică este limitată la ≥75 ani. **Insuficiență renală:** Suliqua nu este recomandat la pacienții cu insuficiență renală severă și cu afecțiune renală în stadiu terminal. Nu este necesară ajustarea dozei de lixisenatidă la pacienții cu insuficiență renală ușoară sau moderată. La pacienții cu insuficiență renală, necesarul de insulină poate fi diminuat și pot fi necesare monitorizarea frecventă a glicemiei și ajustarea dozei. **Insuficiență hepatică:** Nu este necesară ajustarea dozei de lixisenatidă. Necesarul de insulină poate fi diminuat și pot fi necesare monitorizarea frecventă a glicemiei și ajustarea dozei. **Copii și adolescenți:** Suliqua nu prezintă utilizare relevantă la copii și adolescenți. **Mod de administrare:** Suliqua trebuie injectat subcutanat, la nivel abdominal, deltoidian sau al coapsei. Locurile de injectare trebuie alternate în cadrul aceleiași regiuni (abdomen, deltoid sau coapsă) de la o injecție la alta, pentru a scădea riscul de lipodistrofie și amiloidoză cutanată. Pacienții trebuie instruiți să utilizeze întotdeauna un ac nou. Suliqua nu trebuie extras cu seringă din cartușul stiloului injector (pen-ului) preumplut, pentru a evita erorile de administrare și un potențial supradozaj. Vezi RCP complet pentru toate informațiile. **Contraindicații:** Hipersensibilitate la substanțele active sau la oricare dintre excipienți. **Atenționări și precauții:** Pentru a avea sub control trasabilitatea medicamentelor biologice, numele și numărul lotului medicamentului administrat trebuie înregistrate cu atenție. Suliqua nu trebuie utilizat la pacienții cu diabet zaharat de tip 1 sau pentru tratamentul cetoacidozei diabetice. Pacienții trebuie instruiți să alterneze continuu locurile de injectare, pentru a reduce riscul de apariție a lipodistrofiei și amiloidozei cutanate. Există un posibil risc de absorbție întârziată a insulinei sau de reglare insuficientă a glicemiei în urma injectării insulinei în locuri unde au apărut aceste reacții. Schimbarea bruscă a locului de injectare cu o zonă neafectată duce la hipoglicemie. Se recomandă monitorizarea glicemiei după schimbarea locului de injectare și se poate avea în vedere ajustarea dozei de medicament antidiabetic. Hipoglicemia poate apărea dacă doza de Suliqua este mai mare decât este necesar; doza trebuie stabilită individual, pe baza răspunsului clinic. Au fost raportate câteva evenimente de pancreatită acută pentru lixisenatidă, cu toate că nu a fost stabilită o relație de cauzalitate. Pacienții trebuie informați despre simptomele caracteristice: durere abdominală severă, persistentă. Dacă este suspectată pancreatita, trebuie întrerupt tratamentul cu Suliqua; dacă pancreatita acută se confirmă, nu trebuie reîncepută administrarea lixisenatidei. Este necesară prudență la pacienții cu antecedente de pancreatită. Suliqua nu este recomandat la pacienții cu afecțiuni gastro-intestinale severe, inclusiv gastropareză severă. Este necesară precauție la pacienții tratați cu medicamente administrate oral care necesită o absorbție gastro-intestinală rapidă, supraveghere clinică atentă sau care au un indice terapeutic îngust (pentru detalii, vezi pct. 4.5 din RCP complet). Sfătuți pacienții să ia măsuri de precauție pentru a se evita depleția de lichide. În cazuri rare, prezența de anticorpi poate face necesară ajustarea dozei de Suliqua. Pacienții trebuie instruiți să verifice întotdeauna eticheta stiloului injector (pen-ului) înainte de fiecare injecție. Suliqua nu a fost studiat în asociere cu inhibitori ai dipeptidil peptidazei 4, medicamente sulfonilureice, glinide, pioglitazonă. Acest medicament conține metacrezol, care poate provoca reacții alergice. **Interacțiuni:** Nu s-au efectuat studii privind interacțiunile cu Suliqua. Informațiile prezentate în RCP complet se bazează pe studii cu fiecare componentă în parte (vezi pct. 4.5 din RCP complet pentru toate detaliile). **Fertilitatea, sarcina și alăptarea:** Nu este recomandat la femeile aflate la vârsta fertilă care nu utilizează măsuri de contracepție. Suliqua nu trebuie utilizat în timpul sarcinii sau al alăptării. **Efecte asupra capacității de a conduce vehicule:** Suliqua nu are nicio influență sau are influență neglijabilă asupra capacității de a conduce vehicule sau de a folosi utilaje. Pacienții trebuie sfătuiți să-și ia măsuri de precauție pentru a evita hipoglicemia în timp ce conduc vehicule și folosesc utilaje. **Reacții adverse:** Reacțiile adverse raportate cel mai frecvent în timpul tratamentului cu Suliqua au fost hipoglicemia și reacțiile adverse gastro-intestinale. Din studii clinice: Foarte frecvente: hipoglicemie. Frecvente: amețeli, greață, diaree, vărsături, reacții la nivelul locului de injectare. Pentru reacțiile adverse apărute mai puțin frecvent, rar, foarte rar sau cu frecvență necunoscută, citiți RCP complet. Pentru modul de raportare a reacțiilor adverse, vezi www.anm.ro/Raportează o reacție adversă. **DAPP:** sanofi-aventis groupe **Mod de eliberare:** pe bază de prescripție medicală PRF. **Data primei autorizări:** 11 ianuarie 2017. **Text revizuit la data de:** ianuarie 2021, bazat pe RCP revizuit în iulie 2020. Pentru informații complete de prescriere, citiți Rezumatul caracteristicilor produsului.

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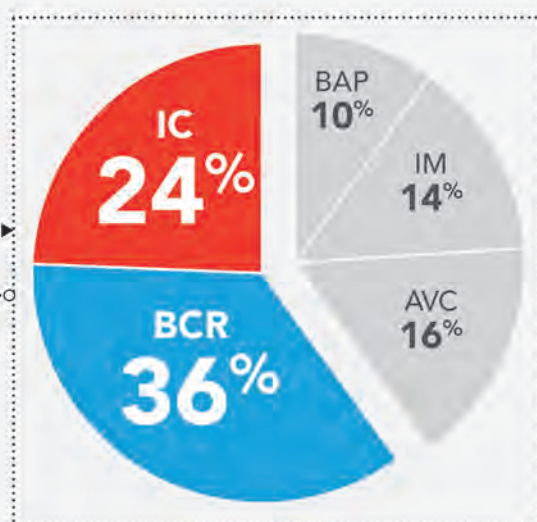
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EMPEROR-PRESERVED, PRIMUL STUDIU CLINIC PENTRU INSUFICIENȚĂ CARDIACĂ CU FRAȚIE DE EJECTIE PĂSTRATĂ CE ȘI-A ATINS OBIECTIVUL



Rezultatele deosebite ale empagliflozin confirmă EMPEROR-Preserved ca fiind primul și singurul studiu clinic pentru insuficiență cardiacă cu fracție de ejecție păstrată care și-a atins obiectivul.

► Studiul clinic de fază III EMPEROR-Preserved și-a atins obiectivul primar și a demonstrat o reducere semnificativă a riscului combinat de mortalitate cardiovasculară și spitalizare pentru insuficiență cardiacă la pacienții cu insuficiență cardiacă cu fracție de ejecție păstrată (IC-FEp), la administrarea de empagliflozin.

► IC-FEp a fost clasificată ca fiind “principala nevoie neacoperită în medicina cardiovasculară” luând în considerare prevalența, efectele nefavorabile ale patologiei și absența până în prezent a tratamentelor aprobate clinic.

► Odată aprobat, empagliflozin va deveni primul și singurul tratament clinic dovedit că oferă rezultate superioare pentru întregul spectru de pacienți cu insuficiență cardiacă, indiferent de fracția de ejecție.

Ingelheim, Germania și Indianapolis, SUA, 6 iulie 2021 – Studiul de fază III EMPEROR-Preserved și-a atins obiectivul principal, poziționând empagliflozin ca primul și singurul tratament care reduce semnificativ riscul combinat de mortalitate cardiovasculară și spitalizare la adulții cu insuficiență cardiacă, cu sau fără diabet zaharat, care suferă de insuficiență cardiacă cu fracție de ejecție păstrată (IC-FEp). Boehringer Ingelheim a dezvăluit astăzi rezultatele preliminare. Împreună cu rezultatele studiului EMPEROR-Reduced, acestea demonstrează eficacitatea empagliflozin pentru toate formele de insuficiență cardiacă, indiferent de fracția de ejecție. Profilul de siguranță general a fost în conformitate cu profilul de siguranță deja cunoscut al empagliflozin.

“Suntem nerăbdători să prezentăm rezultatele studiului EMPEROR-Preserved la ESC 2021, care ar trebui să deschidă noi orizonturi în domeniul medicinei cardiovasculare și să ofere o nouă speranță pacienților care suferă de IC-FEp, care reprezintă o problemă de sănătate publică din ce în ce mai des întâlnită. IC-FEp a constituit pentru mult timp cea mai greu de tratat formă de insuficiență cardiacă,” a spus Stefan Anker, profesor universitar și cardiolog specializat în insuficiență cardiacă în cadrul Charité Berlin, Germania, și investigator principal în studiul EMPEROR-Preserved. “Folosindu-ne de rezultatele anterioare ale studiului clinic EMPA-REG-OUTCOME și ale studiului EMPEROR-Reduced pentru insuficiență cardiacă cu fracție de ejecție redusă, rezultatele studiului EMPEROR-Preserved demonstrează că empagliflozin reduce mortalitatea cardiovasculară sau spitalizarea în cazul insuficienței cardiace și are potențialul de a transforma major îngrijirea medicală oferită pacienților cu insuficiență cardiacă.”

Insuficiența cardiacă reprezintă o problemă clinică semnificativă la nivel mondial. Peste 60 de milioane de pacienți din toată lumea suferă de insuficiență cardiacă, iar jumătate dintre aceștia au IC-FEp. Insuficiența cardiacă este principala cauză de spitalizare și este din ce în ce mai întâlnită în țările vestice din cauza îmbătrânirii demografice.³ Riscul de deces la pacienții cu insuficiență cardiacă crește proporțional cu cel al internărilor în spital. Insuficiența cardiacă cu fracție de ejecție păstrată a ventriculului stâng survine atunci când ventriculul stâng nu poate să se umple corespunzător, rezultând într-o cantitate mai mică de sânge pompat în organism.

“Totalitatea evidențelor din studiu indică potențialul empagliflozin de a redefini tratamentul insuficienței cardiace cu fracție de ejecție păstrată, care afectează aproximativ 30 de milioane de pacienți la nivel mondial,” a declarat Waheed Jamal,

M.D., vice-președinte al companiei și directorul departamentului de Medicină Cardio-metabolică din cadrul Boehringer

Ingelheim. “Până în prezent, nu au existat studii clinice care să demonstreze o reducere a riscului de internare în spital din cauza insuficienței cardiace sau a riscului de mortalitate cardiovasculară la totalitatea pacienților adulți care suferă de această boală prevalentă și care reduce durata vieții. Cumulate cu rezultatele anterioare din cadrul studiului EMPEROR-Reduced, suntem încrezători în potențialul empagliflozin de a avea un impact pozitiv asupra vieții pacienților adulți care se încadrează în întreg spectru de insuficiență cardiacă.”

Studiul clinic EMPEROR-Preserved a investigat empagliflozin 10 mg în comparație cu placebo. Rezultatele complete ale studiului clinic EMPEROR-Preserved urmează să fie prezentate în cadrul Congresului Societății Europene pentru Cardiologie 2021 în data de 27 august. Datele vor fi incluse în planul de obținere a indicației și vor fi înaintate către agențiile de reglementare în 2021.

Aceste rezultate vin în completarea rezultatelor anterioare din cadrul studiului clinic de fază III EMPEROR-Reduced, care a demonstrat că empagliflozin a redus semnificativ, cu 25% riscul de mortalitate cardiovasculară sau spitalizare pentru pacienții cu insuficiență cardiacă, în comparație cu placebo în cazul pacienților adulți cu insuficiență cardiacă cu fracție redusă de ejecție (IC-FEr). Împreună, aceste studii au demonstrat beneficiile aduse de empagliflozin pentru pacienții din întregul spectru al insuficienței cardiace (incluzând IC-FEp și IC-FEr).

Rezultatele studiului clinic EMPEROR-Reduced au creat baza aprobării recente de către Comisia Europeană a unei noi indicații terapeutice pentru empagliflozin, pentru tratarea pacienților adulți cu IC-FEr. O solicitare suplimentară pentru medicament nou (supplemental New Drug Application - sNDA) a fost înaintată către Administrația pentru Medicamente și Alimente din SUA (FDA) pentru empagliflozin pentru reducerea riscului de mortalitate cardiovasculară sau spitalizare cauzate de insuficiență cardiacă la pacienții adulți cu IC-FEr, iar o decizie este așteptată pe parcursul acestui an. Empagliflozin este în prezent indicat pentru tratamentul pacienților adulți cu diabet tip 2 insuficient controlat (și suplimentar în UE, pentru tratamentul pacienților adulți cu IC-FEr).^{5,6} Se derulează cercetări pentru a se determina efectul empagliflozin asupra internărilor în spital în cazul insuficienței cardiace și asupra mortalității în cazul pacienților care cu infarct post-miocardic (atac de cord) cu risc crescut de insuficiență cardiacă. Empagliflozin este studiat în prezent și pentru boală renală cronică.

Despre studiile EMPEROR în Insuficiența Cardiacă^{2,4,6}

Studiile EMPEROR (EMPagliflozin outcome tRial in patients with chrOnic heaRt failure) în insuficiența cardiacă sunt două studii dublu-orb, randomizate, de fază III, care au investigat siguranța și eficacitatea empagliflozin administrat o dată pe zi, comparativ cu placebo, la pacienți adulți cu insuficiență cardiacă cu fracție de ejecție păstrată sau redusă*, atât cu diabet cât și fără diabet, care primesc tratament standard conform ghidurilor în vigoare: ► **EMPEROR-Reduced [NCT03057977]** a investigat siguranța și eficacitatea empagliflozin la pacienții cu insuficiență cardiacă cronică cu **fracție de ejecție redusă (HFrEF - heart failure with reduced ejection fraction).**

- Obiectiv primar: timpul până la apariția primului eveniment adjudecat de deces de cauză cardiovasculară sau eveniment adjudecat de spitalizare pentru insuficiență cardiacă
- Număr de pacienți: 3.730
- Finalizare: 2020

► **EMPEROR-Preserved** [NCT03057951] investighează siguranța și eficacitatea empagliflozin la pacienții cu insuficiență cardiacă cronică cu **fracție de ejeție păstrată (HFpEF - heart failure with preserved ejection fraction)**.

- Obiectiv primar: timpul până la apariția primului eveniment adjudecat de deces de cauză cardiovasculară sau eveniment adjudecat de spitalizare pentru insuficiență cardiacă
- Număr de pacienți: aproximativ 5.988
- Finalizare estimată: 2021

Fracția de ejeție este un indicator al procentului de sânge pompat de ventriculul stâng la fiecare contracție.¹⁷ Când inima se relaxează, ventriculul se reumple cu sânge.

Despre programul EMPOWER

Boehringer Ingelheim a dezvoltat programul EMPOWER pentru a investiga impactul empagliflozin asupra obiectivelor clinice cardiovasculare și renale majore într-o serie de afecțiuni cardio-reno-metabolice. Afecțiunile cardio-reno-metabolice reprezintă principala cauză de mortalitate la nivel mondial și sunt responsabile pentru până la 20 de milioane de decese anual.¹⁸ Prin programul EMPOWER, Boehringer Ingelheim dorește să contribuie la progresul cunoștințelor despre aceste sisteme interconectate și să creeze tratamente care să ofere beneficii integrate în ceea ce privește afectarea multiplă de organ. Programul EMPOWER, alcătuit din opt studii clinice și două studii cu dovezi din viața reală, subliniază angajamentul pe termen lung pentru îmbunătățirea prognosticului bolii persoanelor care trăiesc cu afecțiuni cardio-reno-metabolice. Sunt peste 400.000 de adulți înrolați la nivel mondial în aceste studii clinice, acesta fiind unul din cele mai ample și mai cuprinzătoare programe clinice desfășurate până în prezent pentru un inhibitor SGLT2.

Despre insuficiența cardiacă

Insuficiența cardiacă este o afecțiune progresivă, debilitantă și cu potențial letal care apare atunci când inima nu poate asigura o circulație adecvată pentru a îndeplini necesitățile de sânge oxigenat ale organismului sau, pentru a realiza acest lucru, necesită un volum crescut de sânge, ceea ce duce la acumularea de lichid (congestie) în plămâni și țesuturile periferice.¹⁹ Este o afecțiune extrem de răspândită, care afectează mai mult de 60 de milioane de persoane la nivel global, număr care este preconizat să crească pe măsura îmbătrânirii populației.^{3,4} Insuficiența cardiacă are o prevalență ridicată la persoanele cu diabet;²⁰ cu toate acestea, aproximativ jumătate dintre pacienții cu insuficiență cardiacă nu au și diabet.²¹

Despre empagliflozin

Empagliflozin (cu denumirea comercială de Jardiance[®]) este un inhibitor al co-transportorului 2 de sodiu-glucoză (SGLT2) cu administrare orală, o dată pe zi, cu selectivitate înaltă și este primul medicament pentru diabetul zaharat tip 2 care include date privind reducerea riscului de deces de cauză cardiovasculară în etichetarea sa din mai multe țări.^{11,12,13}

Despre Boehringer Ingelheim

Boehringer Ingelheim se concentrează pe tratamente inovatoare care să îmbunătățească viața oamenilor și animalelor. În calitate de companie biofarmaceutică de top la nivel mondial, cu activitatea îndreptată spre cercetare, compania crează valoare prin inovație în arii cu nevoi medicale neîmplinite încă. Fondată în 1885 și rămasă o companie de familie, Boehringer Ingelheim are perspective pe termen lung. Aproximativ 52 000 de angajați din peste 130 de țări își aduc contribuția în fiecare zi, în cele trei domenii de activitate: Medicină Umană, Medicină Veterinară și Producție Biofarmaceutică pe bază de Contract. Informații suplimentare la www.boehringer-ingelheim.com.

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Jardiance® (empagliflozin) comprimate filmate 10 mg, 25 mg. **Indicații terapeutice:** Tratamentul adulților cu diabet zaharat tip 2 insuficient controlat, ca adjuvant la dietă și exercițiul fizic; ca monoterapie atunci când metformina este considerată inadecvată din cauza intoleranței; în plus față de alte medicamente pentru tratamentul diabetului zaharat și tratamentul adulților cu insuficiență cardiacă cronică simptomatică, cu fracție de ejeție redusă. **Doze și mod de administrare:** Diabet zaharat: doza inițială este de 10 mg o dată pe zi (în monoterapie și în terapie asociată suplimentară cu alte medicamente pentru tratamentul diabetului zaharat). La pacienții care tolerează empagliflozin 10 mg o dată pe zi, care prezintă RFGe ≥ 60 ml/min/1,73 m² și care necesită un control glicemic mai strict, doza poate fi crescută la 25 mg o dată pe zi. Insuficiență cardiacă: doza recomandată este 10 mg o dată pe zi. Când empagliflozin este utilizat în asociere cu o sulfoniluree sau cu insulină, se poate avea în vedere o doză mai mică de sulfoniluree sau de insulină, pentru a reduce riscul de hipoglicemie. IR. Diabet zaharat: Nu este necesară ajustarea dozei la pacienți cu RFGe ≥ 60 ml/min/1,73 m² sau ClCr ≥ 60 ml/min. Administrarea nu trebuie inițiată la pacienți cu RFGe < 60 ml/min/1,73 m² sau ClCr < 60 ml/min. Insuficiență cardiacă: la pacienții cu sau fără diabet zaharat de tip 2, administrarea de empagliflozin 10 mg poate fi inițiată sau continuată până la o valoare a RFGe de 20 ml/minut și 1,73 m² sau a ClCr de 20 ml/minut. Nu trebuie utilizat la pacienții cu BRST sau cărora li se efectuează dializă. IH Nu este necesară ajustarea dozei la pacienți cu IH. Nu se recomandă utilizarea la pacienți cu IH severă. Vârstnici La pacienții cu vârsta de 75 ani și peste, trebuie avut în vedere un risc crescut de depleție volemică. Nu se recomandă începerea tratamentului cu empagliflozin la pacienții cu vârsta de 85 ani și peste. Copii și adolescenți La acest grup de pacienți, siguranța și eficacitatea administrării empagliflozin nu au fost încă stabilite. Nu sunt disponibile date. Mod de administrare Comprimatele pot fi administrate cu sau fără alimente, înghițite întregi cu apă. Dacă se omite o doză, aceasta trebuie administrată imediat ce pacientul își aduce aminte. În aceeași zi nu trebuie administrată o doză dublă. **Contraindicații:** Hipersensibilitate la substanța activă sau la oricare dintre excipienți. **Atenționări și precauții speciale pentru utilizare:** Au fost raportate cazuri rare de CAD, inclusiv cazuri cu risc vital și cazuri letale la pacienți tratați cu inhibitori de SGLT2, inclusiv empagliflozin. La pacienții unde se suspectează sau este diagnosticată CAD, tratamentul trebuie întrerupt imediat. *Jardiance* nu trebuie utilizat pentru tratarea pacienților cu diabet zaharat de tip 1. Datele provenite dintr-un program de studii clinice privind diabetul zaharat de tip 1 au arătat o creștere a incidenței CAD, care apare frecvent la pacienții cărora li se administrează empagliflozin 10 mg și 25 mg ca adjuvant la insulină, comparativ cu placebo. Monitorizarea funcției renale: Înainte de începerea tratamentului și periodic în timpul tratamentului, cel puțin anual; Înainte de începerea tratamentului concomitent cu orice medicament care poate avea impact negativ asupra funcției renale. În insuficiență cardiacă *Jardiance* nu este recomandat la pacienții cu valori ale RFGe < 20 ml/minut și 1,73 m². Leziuni hepatice Cazuri de leziuni hepatice au fost raportate atunci când s-a administrat empagliflozin în cadrul studiilor clinice. Nu s-a stabilit o relație de cauzalitate între empagliflozin și leziunea hepatică. Risc de depleție volemică Se impune prudență la pacienții la care scăderea tensiunii arteriale indusă de empagliflozin ar putea prezenta un risc. Până la corectarea pierderii de lichide, se va avea în vedere întreruperea temporară a tratamentului cu empagliflozin. Infecții complicate ale căilor urinare La pacienții cărora li s-a administrat tratament cu empagliflozin s-au raportat ulterior punerii pe piață cazuri de infecții complicate ale căilor urinare. Fasceită necrozantă care afectează perineul (gangrena Fournier) După punerea pe piață, s-au raportat cazuri de fasceită necrozantă care afectează perineul (afecțiune cunoscută și ca gangrena Fournier) la pacienți de ambele sexe tratați cu inhibitori de SGLT2. Este un eveniment rar, însă grav, care poate pune în pericol viața și care necesită intervenție chirurgicală urgentă și tratament cu antibiotice. În cazul în care există suspiciunea de gangrenă Fournier, se va întrerupe administrarea *Jardiance* și se va institui imediat tratament (inclusiv antibiotice și debridare chirurgicală). Evaluarea analizelor de laborator ale urinei Pacienții care urmează tratament cu *Jardiance* vor avea glicozurie pozitivă. Lactoză Pacienții cu afecțiuni ereditare rare de intoleranță la galactoză, deficit de lactază Lapp sau sindrom de malabsorbție a glucozei-galactozei nu trebuie să utilizeze acest medicament. **Fertilitatea, sarcina și alăptarea:** Este de preferat să se evite utilizarea *Jardiance* în timpul sarcinii. Nu trebuie utilizat în timpul alăptării. Studiile la animale nu au evidențiat efecte dăunătoare directe sau indirecte cu privire la fertilitate. **Efecte asupra capacității de a conduce vehicule și de a folosi utilaje:** Pacienții trebuie sfătuiți să ia măsuri de precauție pentru a evita hipoglicemia atunci când conduc vehicule și folosesc utilaje, în special atunci când *Jardiance* este utilizat în asociere cu o sulfoniluree și/sau insulină. **Reacții adverse (RA):** Diabet zaharat de tip 2 Siguranța administrării empagliflozinului a fost evaluată la un număr total de 15582 pacienți cu diabet zaharat de tip 2 incluși în studiile clinice. Reacția adversă raportată cel mai frecvent a fost hipoglicemia, atunci când se asociază cu sulfoniluree sau insulină. Insuficiență cardiacă Studiul EMPEROR-Reduced a inclus 3730 pacienți cu insuficiență cardiacă și fracție de ejeție redusă tratați cu empagliflozin 10 mg sau placebo. Aproximativ jumătate dintre pacienți aveau diabet zaharat de tip 2. Cea mai frecventă reacție adversă a fost depleția volemică. Hipoglicemia severă a fost observată numai la pacienții cu diabet zaharat. Foarte frecvente: hipoglicemie (atunci când se utilizează împreună cu sulfoniluree sau insulină), depleție volemică. RA frecvente: Candidoză vaginală, vulvovaginită, balanită infecții ale căilor urinare, sete, constipație, prurit, erupție cutanată tranzitorie, diureză crescută, creștere a lipidelor serice. RA mai puțin frecvente: urticarie, angioedem, disurie, creșterea creatininei sanguine/scăderea ratei de filtrare glomerulară, creșterea hematocritului. RA rare: CAD, fasceită necrozantă care afectează perineul (gangrena Fournier). **Raportarea reacțiilor adverse suspectate:** Profesioniștii din domeniul sănătății sunt rugați să raporteze orice reacție adversă suspectată prin intermediul sistemului național de raportare ale cărui detalii sunt publicate pe web-site-ul ANMDMR: <http://www.anm.ro>. **Deținătorul Autorizației de Punere Pe Piață:** Boehringer Ingelheim International GmbH, Binger Strasse 173, 55216 Ingelheim am Rhein, Germania. **Data ultimei reînnoiri a autorizației:** 02/2019. **Data revizuirii textului:** 06/2021. *Jardiance®* se eliberează pe bază de prescripție medicală PRF. Acestea reprezintă informații abreviate despre produse. Pentru orice informații despre acest medicament, vă rugăm să contactați reprezentanții locali ai deținătorului autorizației de punere pe piață sau să consultați rezumatul caracteristicilor produsului complet. Acest material promoțional este destinat profesioniștilor din domeniul sănătății.





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Referințe: 1. JARDIANCE Rezumatul Caracteristicilor Produsului, iunie 2021 2. Zinman B, Inzucchi S, Lachin JM, et al. EMPA-REG OUTCOME[®] Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin. Cardiovasc Diabetol. 2014;13(102):1-8.

Acest material este destinat profesioniștilor în domeniul sănătății. PC-RO-100528

O SOLUȚIE PENTRU OBTINEREA CONTROLULUI GLICEMIC LA PACIENȚII DUMNEAVOASTRĂ CU DIABET ZAHARAT TIP 2



Acest material promoțional este destinat profesioniștilor din domeniul sănătății. Acest medicament se eliberează pe bază de prescripție medicală PRF. Rezumatul Caracteristicilor Produsului, data revizuirii iulie 2020

Acest medicament face obiectul unor măsuri de reducere la minimum a riscului. Pentru informații complete, vă rugăm să citiți în întregime RCP

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